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THE BEHAVIOR OF PAIRED MONOSPOROUS MYCELIA OF *LENZITES SAEPIARIA* (WULF.) FR., *L. TRABEA* (PERS.) FR., *L. THERMOPHILA* FALCK, AND *TRAMETES AMERICANA* OVERH.[1]

BY IRENE MOUNCE AND RUTH MACRAE[2]

Abstract

Lenzites saepiaria, *L. trabea*, and *Trametes americana* are heterothallic and bipolar. In each species complete interfertility exists between haploid mycelia derived from different sources. *T. americana* is sometimes considered to be a pored form of *L. saepiaria*. The failure to obtain clamp connections in any pairing of a haploid mycelium of *L. saepiaria* with a haploid mycelium of *T. americana* seems significant and lends weight to the conclusion that these two forms are distinct. Haploid mycelia of *L. thermophila* are completely interfertile with those of *L. trabea*.

Introduction

Lenzites saepiaria (Wulf.) Fr. and *Trametes americana* Overh. (3) are two species of Polyporaceae which are found commonly on the wood of coniferous trees. The latter is the fungus which until recently has been referred to *T. odorata* (Wulf.) Fr. or to *T. protracta* Fr. by American authors. They resemble one another in shape and in the rusty brown color of pileus and context, but differ in that the hymenial region of *L. saepiaria* is typically lamellate (Plate I, 5) while that of *T. americana* is typically pored (Plate I, 7), although intergrading forms do occur. They are regarded usually as two distinct species, but it has been suggested that *T. americana* is but a pored form of *L. saepiaria*. *L. trabea* is distinct in the more grayish brown color of its context and of its hymenial surface, which is never entirely gilled but shows some elongate pores (Plate I, 1, 2, 3). It usually occurs on the wood of deciduous trees and all three are found on structural timbers.

Snell, Hutchinson and Newton (5) have shown by experiments with cultures of *T. americana* (*T. protracta*) and of *L. saepiaria* that, although their optimum temperature of growth is about the same, there is such a decided difference in both their upper limits of growth and their rates of growth that a test upon a single agar at temperatures of from 30° C. to 36° C. would serve to distinguish these two fungi in culture.

Because of the similarities which do exist, we were interested in studying the behavior of paired monosporous mycelia of *L. saepiaria*, *L. trabea*, and *T. americana* and in applying to them the clamp-connection criterion for identity of species (6).   Preliminary reports have been published in the Report of the Dominion Botanist for 1928, 1929, and 1930.

Isolation of Single Spores

Spores from a moistened fruit-body were allowed to fall directly on sterile lactose-dextrose gelatine in Petri dishes or a spore deposit was obtained from a sporophore produced in culture, mixed with a large drop of sterile distilled water, and smeared over the surface of the sterile gelatine.   After germination, isolations were made by cutting out with a fine needle under the compound microscope a square of gelatine containing a single spore and placing this in a tube of potato-dextrose agar.

Paired Monosporous Mycelia

Lenzites saepiaria

Single basidiospore cultures were obtained from the sources indicated in Table I.

TABLE I

Culture No.	No. of isolations	Host	Locality
835	23	*Abies balsamea*	Timagami, Ont.
854	19	*Pinus Strobus*	Timagami, Ont.
996	14	*Pinus Strobus*	Cranberry Lake, Forest Camp, N.Y.
1655	10	*Pinus Strobus*	Meach Lake, P.Q.
1679	15	*Pseudotsuga taxifolia*	Coombs, B.C.
3250	8	*Picea alba*	Chalk River, Ont.
3252	14	*Pinus Banksiana*	Chalk River, Ont.

No clamp connections were found on any mycelium arising from a single basidiospore of *L. saepiaria* or of *T. americana*, but oidia were sometimes abundant.   Falck (2) has given a detailed account of their development in *L. saepiaria*.

While working at this laboratory Miss D. N. Head isolated sixteen single oidium cultures of *L. saepiaria* and nine of *T. americana*.   These mycelia were used in a large number of pairings but, as was to be expected, they behaved in exactly the same way as the haploid mycelium on which they developed, so that the results need not be given here in detail.

Pairings of monosporous mycelia were made on potato-dextrose agar in Petri dishes. The cultures were incubated for from ten to fourteen days, then examined under the microscope. The results of a series of all possible pairings of ten haploid mycelia of culture No. 996 are shown in Table II where the plus sign indicates the presence of clamp connections and the minus sign their absence.

TABLE II

THE RESULTS OF PAIRING IN ALL POSSIBLE COMBINATIONS TEN MONOSPOROUS MYCELIA OF *Lenzites saepiaria* No. 996

	A				a					
	1	4	6	8	2	3	5	7	9	10
1	−	−	−	−	+	+	+	+	+	+
4	−	−	−	−	+	+	+	+	+	+
6	−	−	−	−	+	+	+	+	+	+
8	−	−	−	−	+	+	+	+	+	+
2	+	+	+	+	−	−	−	−	−	−
3	+	+	+	+	−	−	−	−	−	−
5	+	+	+	+	−	−	−	−	−	−
7	+	+	+	+	−	−	−	−	−	−
9	+	+	+	+	−	−	−	−	−	−
10	+	+	+	+	−	−	−	−	−	−

(rows 1,4,6,8 grouped under A; rows 2,3,5,7,9,10 grouped under a)

TABLE III

THE RESULTS OF PAIRING IN ALL POSSIBLE COMBINATIONS TEN MONOSPOROUS MYCELIA OF *Lenzites saepiaria* No. 1679

	A				a					
	1	6	8	10	2	3	4	5	7	9
1	−	−	−	−	+	+	+	+	+	−
6	−	−	−	−	+	+	−	+	+	+
8	−	−	−	−	+	+	−	−	−	+
10	−	−	−	−	+	−	−	−	−	+
2	+	+	+	+	−	−	−	−	−	−
3	+	+	+	−	−	−	−	−	−	−
4	+	−	−	−	−	−	−	−	−	−
5	+	+	−	−	−	−	−	−	−	−
7	+	+	+	−	−	−	−	−	−	−
9	−	+	+	+	−	−	−	−	−	−

(rows 1,6,8,10 grouped under A; rows 2,3,4,5,7,9 grouped under a)

Similar series of pairings were made with haploid mycelia from cultures No. 835, 854, and 1655. In each case the haploid mycelia could be divided into two groups. Clamp connections were formed in every pairing of a member of one group with a member of the other group. *Lenzites saepiaria* is then heterothallic and bipolar. With culture No. 1679 the results obtained were less uniform as is shown in Table III. This might be accounted for, in part, by the fact that the spores were obtained from a sporophore which was moistened and revived after having been kept in the laboratory for two and a half years. However, even here, the results would indicate that the fungus is heterothallic and bipolar.

Pairings of haploid mycelia from different sources were made as indicated in Table IV.

TABLE IV

Culture No.	Host	Locality	Paired with	Culture No.	Host	Locality
835	*Abies balsamea*	Timagami, Ont.	×	854	*Pinus Strobus*	Timagami, Ont.
835	*Abies balsamea*	Timagami, Ont.	×	996	*Pinus Strobus*	Cranberry Lake Camp, N.Y.
996	*Abies balsamea*	New York	×	1655	*Pinus Strobus*	Meach Lake, P.Q.
3250	*Picea alba*	Chalk River, Ont.	×	3252	*Pinus Banksiana*	Chalk River, Ont.

In a total of 63 pairings, clamp connections were formed in every pairing and Table V is typical.

TABLE V

THE RESULTS OF PAIRING FIVE MONOSPOROUS MYCELIA OF *Lenzites saepiaria* No. 835 WITH FIVE MONOSPOROUS MYCELIA OF *L. saepiaria* No. 854

854 \ 835	11	12	13	14	15
11	+	+	+	+	+
12	+	+	+	+	+
14	+	+	+	+	+
15	+	+	+	+	+
16	+	+	+	+	+

TABLE VI

THE RESULTS OF PAIRING IN ALL POSSIBLE COMBINATIONS TEN MONOSPOROUS MYCELIA OF *Trametes americana* No. 903

		A					a				
		1	4	8	10	11	2	3	5	6	7
A	1	−	−	−	−	−	+	+	+	+	+
	4	−	−	−	−	−	+	+	+	+	+
	8	−	−	−	−	−	+	+	+	+	+
	10	−	−	−	−	−	+	+	+	+	+
	11	−	−	−	−	−	+	+	+	+	+
a	2	+	+	+	+	+	−	−	−	−	−
	3	+	+	+	+	+	−	−	−	−	−
	5	+	+	+	+	+	−	−	−	−	−
	6	+	+	+	+	+	−	−	−	−	−
	7	+	+	+	+	+	−	−	−	−	−

TABLE VII

THE RESULTS OF PAIRING FIVE MONOSPOROUS MYCELIA OF *Trametes americana* No. 990 WITH FIVE MONOSPOROUS MYCELIA OF *T. americana* No. 903

903 \ 990	1	2	6	7	8
2	+	+	+	+	+
4	+	+	+	+	+
6	+	+	+	+	+
8	+	+	+	+	+
10	+	+	+	+	+

Therefore, complete interfertility obtained when pairings were made between haploid mycelia derived from fruit-bodies collected in different localities.

Trametes americana

Single basidiospore cultures were isolated from the sources shown in Table VIII.

TABLE VIII

Culture No.	No. of isolations	Host	Locality
903	10	*Picea canadensis*	Timagami, Ont.
990	8	*Thuja plicata*	Slocan Valley, B.C.
3251	18	*Picea alba*	Chalk River, Ont.
3253	14	*Pinus Banksiana*	Chalk River, Ont.
3254	8	*Pinus Banksiana*	Chalk River, Ont.

Like *L. saepiaria* this fungus is heterothallic and bipolar (Table VI). Haploid mycelia from different sources are mutually interfertile (Table VII) as shown by the results obtained from the pairings of such mycelia given in Table IX.

TABLE IX

Culture No.	Host	Locality	Paired with	Culture No.	Host	Locality
903	*Picea canadensis*	Timagami, Ont.	×	990	*Thuja plicata*	Slocan Valley, B.C.
3251	*Picea alba*	Chalk River, Ont.	×	3253	*Pinus Banksiana*	Chalk River, Ont.
3251	*Picea alba*	Chalk River, Ont.	×	3254	*Pinus Banksiana*	Chalk River, Ont.

In a total of 44 pairings, clamp connections were formed in every pairing (Table VII). These results show clearly that both *L. saepiaria* and *T. americana* are heterothallic and bipolar and in each species complete interfertility exists between haploid mycelia derived from different sources.

At Chalk River, Ontario, Mr. A. W. McCallum and Mr. C. G. Riley collected sporophores of *Lenzites saepiaria* No. 3250 and *Trametes americana* No. 3251 which were growing within three feet of each other on a *Picea alba* log, and sporophores of *L. saepiaria* No. 3252 and *T. americana* No. 3253 on the one *Pinus Banksiana* log. Single basidiospore cultures were isolated from one sporophore in each of these four collections and the pairings were made as shown in Table X.

TABLE X

Culture No.	Fungus	Paired with	Culture No.	Fungus	No. of pairings	Clamp connections Present	Clamp connections Absent
3250	*L. saepiaria* from *Picea*	×	3252	*L. saepiaria* from *Pinus*	8	8	0
3251	*T. americana* from *Picea*	×	3253	*T. americana* from *Pinus*	10	10	0
3250	*L. saepiaria* from *Picea*	×	3251	*T. americana* from *Picea*	8	0	8
3250	*L. saepiaria* from *Picea*	×	3253	*T. americana* from *Pinus*	10	0	10
3252	*L. saepiaria* from *Pinus*	×	3251	*T. americana* from *Picea*	10	0	10
3252	*L. saepiaria* from *Pinus*	×	3253	*T. americana* from *Pinus*	10	0	10

Haploid mycelia of *L. saepiaria* from *Picea* were completely interfertile with those of *L. saepiaria* from *Pinus;* haploid mycelia of *T. americana* from *Picea* were completely interfertile with those of *T. americana* from *Pinus,* but clamp connections were not formed in any of the 38 pairings of a haploid mycelium of *L. saepiaria* with a haploid mycelium of *T. americana.* In view of the complete interfertility obtained in all our pairings of haploid mycelia of *T. americana* from different sources and similar interfertility between haploid mycelia of *L. saepiaria* from different sources the failure to obtain clamp connections in any pairing of a haploid mycelium of *T. americana* with a haploid mycelium of *L. saepiaria* seems significant and lends weight to the conclusion of Snell (6) and others that these two forms are distinct.

Lenzites trabea and *L. thermophila*

Of these two fungi Mr. Cartwright (1) writes as follows: "This fungus (*L. trabea* from Dr. Audrey Richards) has been compared with a culture of *L. thermophila* Falck from the Centraalbureau voor Schimmelcultures, Baarn,

with one received direct from Falck and also with a culture kindly sent to us by Dr. Liese of *Trametes protracta*. The four cultures are identical in every respect." It seemed an interesting case in which to apply the clamp-connection criterion for identity of species.

Single basidiospore cultures were isolated from the following sources:

L. trabea No. 3237. Sporophores which developed on a bench made of *Taxodium distichum* in a greenhouse, Arboretum, Experimental Farm, Ottawa. (Plate I, 3).

L. trabea No. 1681. A culture received from Mr. Cartwright, Princes Risborough, England, and sent to him by Dr. Audrey Richards of the Forest Products Laboratory, Madison, Wis.

L. thermophila No. 1682. A culture received from Mr. Cartwright.

Series of all possible pairings of monosporous mycelia of *L. trabea* No. 1681 and No. 3237 and of *L. thermophila* No. 1682 showed that each fungus was heterothallic and bipolar (Table XI).

TABLE XI

THE RESULTS OF PAIRING IN ALL POSSIBLE COMBINATIONS TEN MONOSPOROUS MYCELIA OF *Lenzites trabea* No. 3237

		A						a		
	1	6	9	10	15	16	18	2	7	12
A { 1	−	−	−	−	−	−	−	+	+	+
6	−	−	−	−	−	−	−	+	+	+
9	−	−	−	−	−	−	−	+	+	+
10	−	−	−	−	−	−	−	+	+	+
15	−	−	−	−	−	−	−	+	+	+
16	−	−	−	−	−	−	−	+	+	+
18	−	−	−	−	−	−	−	+	+	+
a { 2	+	+	+	+	+	+	+	−	−	−
7	+	+	+	+	+	+	+	−	−	−
12	+	+	+	+	+	+	+	−	−	−

TABLE XII

THE RESULTS OF PAIRING FIVE MONOSPOROUS MYCELIA OF *Lenzites trabea* No. 1681 WITH THREE MONOSPOROUS MYCELIA OF *L. thermophila* No. 1682

|	1681				
	1	2	3	4	5
1682 { 1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+

TABLE XIII

THE RESULTS OF PAIRING FIVE MONOSPOROUS MYCELIA OF *Lenzites trabea* No. 3237 WITH TWO MONOSPOROUS MYCELIA OF *L. thermophila* No. 1682

	3237				
	1	2	3	4	5
1682 { 2	+	+	+	+	+
5	+	+	+	+	+

Haploid mycelia of *L. trabea* were paired with haploid mycelia of *L. saepiaria* and *T. americana* from various sources, but though 109 pairings were made, no clamp connections were formed in any pairing; but clamp connections were formed in every pairing of a haploid mycelium of *L. trabea* No. 1681 and *L. trabea* No. 3237 showing that they were mutually interfertile, and in every pairing of a haploid mycelium of *L. thermophila* with a haploid mycelium from either of the *L. trabea* cultures (Tables XII and XIII). This would indicate that by the clamp-connection criterion *L. trabea* and *L. thermophila* No. 1682 are one and the same species, and corroborates Mr. Cartwright's conclusion.

PLATE I
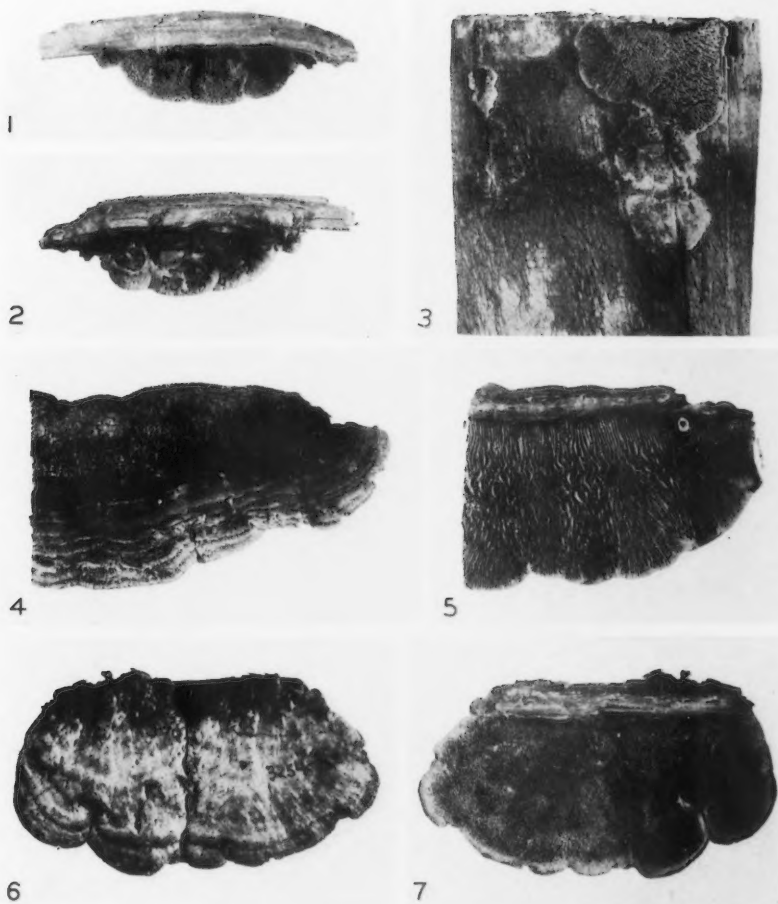
FIGS. 1-2. *Lenzites trabea* No. 5369 showing hymenial surface and pileus. FIGS. 3-7. Sporophores from which spores for experiments were obtained. FIG. 3. *L. trabea* No. 3237. FIGS. 4-5. Upper and lower surface of *L. saepiaria* No. 3250. FIGS. 6-7. Upper and lower surface of *Trametes americana* No. 3254. Three-fourths natural size.
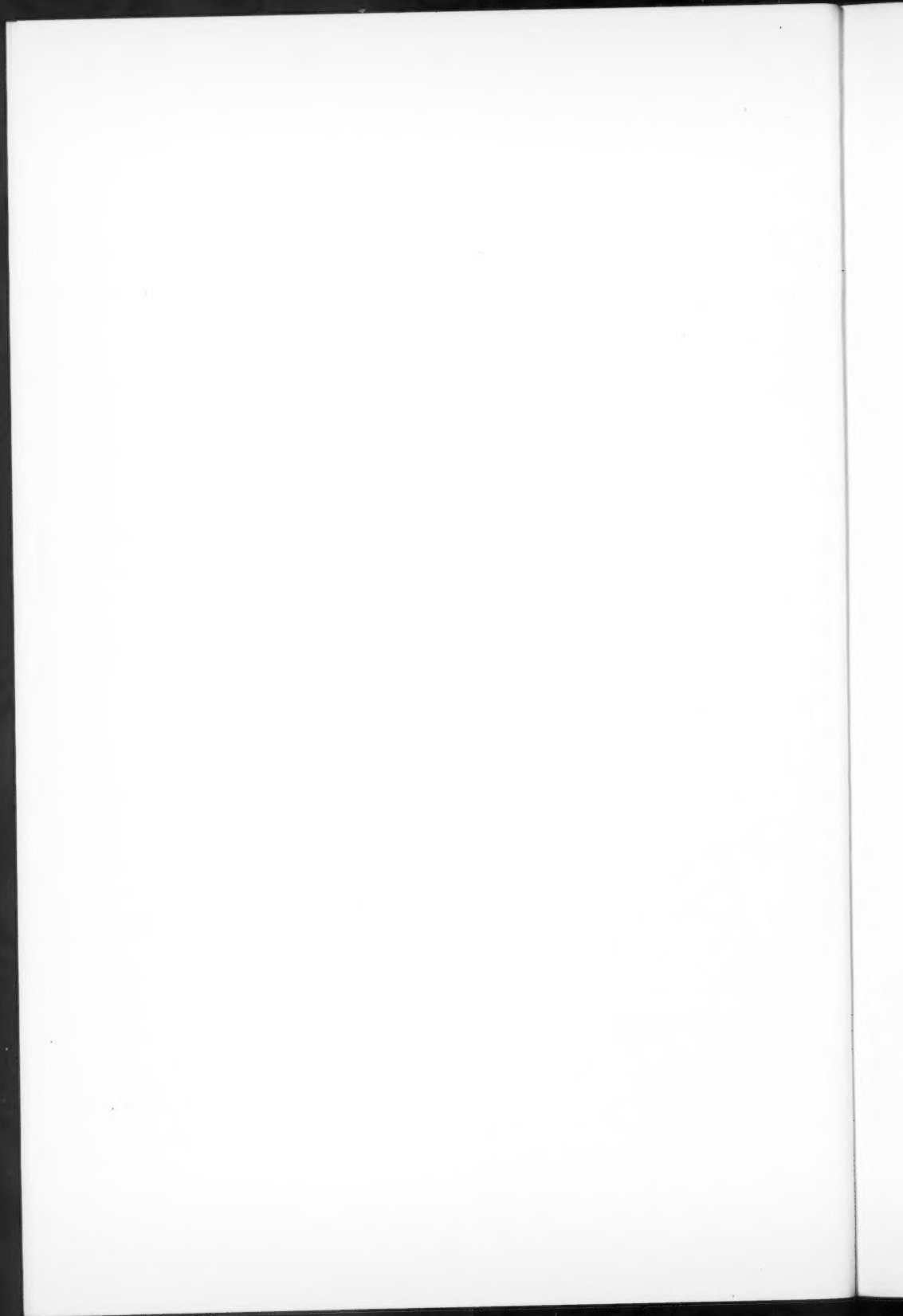
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THE EFFECT OF FERMENTATION ON SOME PROPERTIES OF GLUTEN[1]

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Abstract

Except in the cases of a flour of unusually high protein content (19%) and a Pacific Club wheat flour of 7.6% protein, the loss of gluten protein after four hours' fermentation was about 17% and the change in water-holding capacity was negligible. There was practically no differentiation between flours from heavily frosted wheat and average sound flours. The 19% protein flour, however, showed a loss of gluten protein of only 5% and a slight increase in water-holding capacity; the Club flour showed a loss of 43.4% in gluten protein and a decrease of 27% in water-holding capacity. It is concluded that these two properties of gluten cannot be used to differentiate most flours.

Introduction

During the past decade numerous studies of the relation of wheat and flour protein to baking strength have confirmed the conclusion reached by Zinn (9) in 1923 that these characteristics are significantly and positively correlated. It is unnecessary to discuss again in detail these various investigations; suffice it to state that many workers have concluded, with remarkable unanimity, that protein content of wheat or flour is at present the best single-figure estimate of baking strength. Blish and Sandstedt (2) in discussing the term "flour strength" state that "for all practical purposes, protein content and inherent flour strength are one and the same thing." By the term "flour strength" they refer to the potentialities of the flour rather than to its behavior in any specific baking procedure.

In spite of the general conviction that protein content furnishes a reliable criterion of strength of sound wheat, there is some reluctance to abandon the baking test and it is still common practice to use the results of this test in the final estimate of strength. Many other methods, less time-consuming and presumably more amenable to accurate measurement, have been devised and tried with indifferent success. Of these there might be mentioned the various types of extensimeters, the instruments designed to measure resistance of doughs to mixing, the dough-ball fermentation test, the viscosity test of acidulated flour-water suspensions, and the washed-gluten test. All of these have been devised with a view to getting a figure or figures with which to predict how the flour is likely to behave when subjected to the baking procedure.

The washed-gluten test is the oldest of the short methods of appraising flour characteristics, and it requires the least equipment. There is an exceedingly high positive correlation between dry gluten and protein, as determined by the Kjeldahl method, and if it were as accurate as the latter it would

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be a superior method, because one can determine not only the quantity, but also to some extent the quality, from consideration of the "feel" of the gluten mass as it is being worked from the dough. This test is very useful when applied by a skilled technician, but unfortunately it is difficult to standardize the procedure sufficiently to achieve good agreement among the results obtained by various individuals. This, however, is not sufficient reason for discarding it altogether. The Kjeldahl test, while very accurate, often leads to inaccurate conclusions. Flours of the same protein content, which might be predicted to possess the same baking characteristics, frequently prove quite diverse. Those that fall below expectation are considered of poor quality. In this connection Blish and Sandstedt (2) have made the excellent suggestion that the term "weak" should be taken to indicate the inability of a flour to bake to the standard predicted from its protein content. If a flour fails, when tested by all available means, to come up to the expectations based on its protein content, it must be assumed that the protein has been damaged by adverse conditions of climate or of storage after harvesting, or that it is inherently inferior. In either case prediction equations applicable to normal protein would be invalid.

The weakness might be attributed to inability of the protein to absorb and hold enough water, or the converse, or to a tendency to disperse too readily during fermentation, or to many other causes. In any case, the fact is that the weak flour responds differently to the effect of fermentation than the normal flour of the same protein content. If one were interested in gaining more information regarding the differences between weak and normal flours, it would seem logical to examine the gluten before and after fermentation to determine whether some measurable characteristic was capable of providing an explanation for the differences in baking behavior.

Several workers have approached this problem along these lines. Sharp and Gortner (7) observed that the character of the gluten changed after the first hour of fermentation. Towards the end of the washing period the gluten ball showed a tendency to disintegrate and it was impossible to avoid losses of gluten from this cause. Although the quantity of gluten obtainable decreased, the hydration increased. After eight hours' fermentation no gluten could be separated by washing with distilled water, but if 1% sodium chloride solution was used, gluten could be isolated after 24 hours' fermentation. Sharp and Schreiner (8), using fractionation analysis of the gluten, were unable to find any marked changes resulting from fermentation. Johnson and Green (5) found that the viscosity of flour-water suspensions showed marked decrease as a result of fermentation. Brownlee and Bailey (3) observed that saline peptization of doughs fermented for various periods indicated no change in the proteins as fermentation progressed and concluded that no chemical changes had been effected. They suggested that the progressive increase in acidity as fermentation proceeds causes gradual changes in the dough that affect the imbibitional capacities, and this might account for the changes in viscosity observed by Johnson and Green (5).

The fact that solubility in various solvents appears to be unaffected by fermentation makes it seem unlikely that any important chemical change, such as partial hydrolysis, occurs.   On the other hand, if the idea of McCalla and Rose (6) that gluten is a protein complex rather than a mixture is correct, the failure to obtain gluten after fermentation would suggest some sort of hydrolysis.   In the study herein reported attention was confined to two properties of gluten, namely, the quantity procurable and its water content.

Material and Methods

The flours selected for this investigation are described in Table I.   They were all experimentally milled except two, namely, No. 4, a hard red spring Canadian commercial patent, and No. 5, a Pacific Club commercial patent. Samples No. 17, 18 and 19 were procured by compositing experimentally milled flours from commercial samples of Grades No. 4 Northern, No. 5 and No. 6.

TABLE I

DESCRIPTION AND ANALYSIS OF FLOURS USED

Flour No.	Origin	Moisture, %	Protein (13.5% moisture basis)	Protein (dry basis)
1	Saskatoon composite, Marquis	12.6	19.2	21.97
2	Saskatoon composite, Marquis	12.5	17.9	20.46
3	Saskatoon composite, Marquis	12.6	16.2	18.54
4	Commercial patent	12.9	13.7	15.73
7	Saskatoon composite, Marquis	12.3	13.0	14.82
11	Garnet, composite	12.2	13.2	15.03
16	Winnipeg composite, Marquis, Frosted, Grade 3	12.8	13.1	15.02
17	Winnipeg composite, Marquis, Frosted, Grade 4	12.7	13.4	15.35
78	Saskatoon composite, Marquis	12.1	11.9	13.55
18	Winnipeg composite, Marquis, Frosted, Grade 5	12.7	11.9	13.63
19	Winnipeg composite, Marquis, Frosted, Grade 6	12.8	11.7	13.42
5	Pacific Club	12.5	7.7	8.8

The glutens were washed from doughs containing 25 gm. flour, 5% yeast, 6% sugar and 1% sodium chloride.   Washing was done by hand with tap water at 25° C. flowing at the rate of 110 cc. per minute.   In all instances the washing time was 12 minutes.   Before weighing, the gluten was thoroughly kneaded again, shaped into a small ball, and finally pressed between sheets of filter paper.   While the method of Dill and Alsberg (4) is admitted to be superior to this, it is undoubtedly less convenient and, as only comparative results were desired, the simplest method was chosen.   Further simplification of procedure was achieved by dispensing with immersion of the dough ball for one hour before washing and of the gluten for one hour after washing.   Berliner and Koopmann (1) considered the former unnecessary, and in the present study it was impossible to use it except with unleavened doughs.

As a preliminary, one of us washed many glutens from a stock flour, until sufficient skill was achieved to replicate results on the same day and on different days. After that, the regular method, which involves immersion of dough and gluten, was compared with the proposed shorter method. The results of this comparison are given in Table II. They indicate that more uniform results are obtainable with the shorter method and that the average values by the two methods do not vary significantly.

TABLE II

WEIGHTS OF WET AND DRY GLUTEN OBTAINED FROM 25 GM. FLOUR No. 4 (DRY BASIS) IN 12 REPLICATIONS USING TWO DIFFERENT PROCEDURES

	SHORT PROCEDURE			STANDARD PROCEDURE	
Experiment No.	Weight of wet gluten, gm.	Weight of dry gluten, gm.	Experiment No.	Weight of wet gluten, gm.	Weight of dry gluten, gm.
1	13.49	4.26	1	13.27	4.31
2	13.93	4.57	2	13.44	4.27
3	13.96	4.62	3	13.84	4.49
4	13.57	4.52	4	13.65	4.44
5	13.43	4.45	5	13.65	4.32
6	13.43	4.42	6	13.70	4.39
7	13.69	4.32	7	13.49	4.44
8	13.61	4.40	8	13.81	4.35
9	13.53	4.35	9	13.94	4.47
10	13.84	4.54	10	13.74	4.43
11	13.68	4.41	11	13.43	4.26
12	13.40	4.37	12	13.97	4.54
Total	163.56	53.23		163.93	52.71
Average	13.63 ± .0362	4.44 ± .0201		13.66 ± .0454	4.39 ± .0168
Range	13.40 – 13.96	4.26 – 4.62		13.27 – 13.97	4.26 – 4.54
Standard deviation, σ	.1861	.1034		.2082	.0865

The Effect of Fermentation on the Quantity of Gluten Protein

In Table III there are given the quantities of dry gluten obtained from doughs fermented for four hours at 30° C. and the checks which were obtained from similar doughs without fermentation. There are also given the protein contents of these glutens and various calculated values.

In all cases the actual quantity of dry gluten decreased as a result of fermentation. The dry gluten, however, is not a reliable figure on which to base comparisons because the protein content of glutens varies considerably, no matter what care is taken in the washing. A more reliable figure is the quantity of gluten protein. This value is obtained by multiplying the weight of dry gluten by its protein content as determined by the Kjeldahl method. For convenience this value has been expressed as percentage of flour. Decreases of this value resulting from the four-hour fermentation must be attributed to hydrolysis or to peptization of part of the gluten protein. From column 9, Table III, it can be observed that the percentage decreases were appreciable except with flour No. 1 which was of exceptionally high protein content.

TABLE III

THE EFFECT OF FERMENTATION ON THE QUANTITY OF GLUTEN PROTEIN OBTAINED FROM
TWELVE DIFFERENT FLOURS

Flour No.	Description	Fermentation time, hr.	Total protein in flour, (dry basis), (N×5.7), %	Dry gluten, average of 3 det'ns from 25 gm. flour (dry basis), gm.	Dry gluten as % of flour	Protein in dry gluten (N×5.7) average of 3 det'ns. %	Gluten protein as % of flour	Loss of gluten protein resulting from 4 hr. fermentation, %	Difference between protein in flour and gluten protein in flour expressed as % of total protein in flour
1	Marquis	0	22.0	6.13	24.5	77.8	19.1		13.2
		4		5.26	21.0	86.2	18.1	4.9	17.5
2	Marquis	0	20.5	5.69	22.8	79.8	18.2		11.2
		4		4.60	18.4	86.0	15.8	12.9	22.7
3	Marquis	0	18.5	5.00	20.0	80.9	16.2		12.7
		4		3.93	15.7	86.6	13.6	15.9	26.6
4	Commercial patent	0	15.7	4.21	16.8	77.2	13.0		17.4
		4		3.15	12.6	84.5	10.6	18.1	32.3
17	Frosted 4°	0	15.4	4.04	16.2	83.0	13.4		12.6
		4		3.23	12.9	85.0	11.0	18.2	28.5
11	Garnet	0	15.0	3.92	15.7	84.4	13.2		12.0
		4		3.28	13.1	84.0	11.0	16.6	26.6
16	Frosted 3°	0	15.0	3.99	16.0	81.5	13.0		13.4
		4		3.11	12.4	85.4	10.6	18.3	29.2
7	Marquis	0	14.8	4.07	16.3	80.8	13.2		11.3
		4		3.29	13.2	84.3	11.1	15.6	25.1
18	Frosted 5°	0	13.6	3.45	13.8	83.5	11.5		15.5
		4		2.88	11.5	82.5	9.5	17.5	30.3
78	Marquis	0	13.6	3.37	13.5	84.3	11.4		16.1
		4		3.01	12.0	81.1	9.8	14.1	27.9
19	Frosted 6°	0	13.4	3.32	13.3	82.8	11.0		18.0
		4		2.89	11.6	82.3	9.5	13.4	29.1
5	Pacific Club	0	8.8	2.10	8.4	79.6	6.7		24.0
		4		1.22	4.9	77.7	3.8	43.4	56.9

Flour No. 5, from Pacific Club, gave an enormous decrease amounting to 43.4%. All the others gave decreases ranging from 12.9% to 18.3%. These decreases do not appear to be closely associated with the strength of the flours. For instance, Nos. 78 and 19, of nearly the same protein content but from wheat of very different commercial grades, gave practically the same percentage decrease, although No. 19 was milled from wheat of grade No. 6, severely damaged by frost. Again flour No. 11, milled from Garnet wheat, showed less dispersion than flour No. 4, the commercial hard red spring patent. The only important differentiation in this series in regard to percentage of gluten protein dispersed as a result of fermentation is found with

flours No. 1 and 5. With flour No. 5 it was difficult to wash out the gluten after four hours' fermentation and the great decrease may therefore be accounted for in part as owing to loss in washing. This loss in washing is of course the result of some change in the protein during fermentation. This property of gluten does not seem to offer much promise as a means for differentiating flours of the same class.

The Effect of Fermentation on the Water-holding Capacity of Crude Gluten

The water contained in the glutens from leavened doughs before and after four hours' fermentation was calculated as a percentage on the basis of dry crude gluten and also dry gluten protein. These data, which are given in Table IV, show that in most cases there is not much change in water-holding capacity as a result of fermentation. Exceptions to this are seen in Flours 1,

TABLE IV

THE EFFECT OF FERMENTATION ON THE WATER-HOLDING CAPACITY OF THE GLUTEN PROTEIN

Flour No.	Description	Ferment-ation time, hr.	Protein in flour, dry basis, $(N \times 5.7)$, %	Gluten protein per 100 gm. flour	Weight of water in gluten from 100 gm. flour (dry basis), gm.	Water as % of crude, dry gluten	Water as % of gluten protein
1	Marquis	0	22.0	19.1	48.6	198	255
		4		18.1	49.5	235	273
2	Marquis	0	20.5	18.2	45.8	201	252
		4		15.8	39.0	212	247
3	Marquis	0	18.5	16.2	40.6	203	250
		4		13.6	32.5	207	239
7	Marquis	0	14.8	13.2	29.0	178	221
		4		11.1	24.7	188	222
78	Marquis	0	13.6	11.4	25.1	186	221
		4		9.8	21.2	176	217
16	Marquis, Frosted 3°	0	15.0	13.0	31.0	195	239
		4		10.6	24.3	196	229
11	Garnet	0	15.0	13.2	30.2	192	228
		4		11.0	24.0	183	217
17	Marquis, Frosted 4°	0	15.4	13.4	31.6	196	236
		4		11.0	25.6	199	234
18	Marquis, Frosted 5°	0	13.6	11.5	26.0	188	225
		4		9.5	21.0	183	222
19	Marquis, Frosted 6°	0	13.4	11.0	22.1	166	201
		4		9.5	19.2	166	201
4	Commercial Patent	0	15.7	13.0	34.9	207	269
		4	.	10.6	26.2	208	246
5	Pacific Club	0	8.8	6.7	18.8	224	281
		4		3.8	7.8	160	206

4 and 5. Flour 1, which was very high in protein, showed an increase, while Flours 4 and 5 showed decreases, the decrease in the latter being very great. It should be noted that the water-holding capacity of the Pacific Club flour before fermentation was higher than that of any of the spring wheat flours, and after fermentation it was lower than any of them except No. 19, which was from severely frosted wheat. It is also of interest that Flour 19 showed no change as a result of fermentation.

It seems evident that the water-holding capacity of glutens is little affected by fermentation except in a few cases. The exceptions noted in this study were (a) a flour of unusually high protein content, which showed an increase, and (b) a flour of very low protein content from Pacific Club wheat, which showed a very marked decrease as a result of four hours' fermentation. It was most astonishing to find that flours from severely frosted wheat, grading No. 5 and No. 6, showed no appreciable decrease in water-holding capacity after fermentation. It should be recalled, too, that these flours exhibited no greater loss of actual gluten than did flours from sound wheats. Of course, the glutens could be readily differentiated by "feel" but as there was no way of recording this property accurately it has not been taken into consideration in this discussion.

We are therefore forced to the conclusion that neither the quantity of gluten nor its water content can be used for differentiating many flours that by other experimental tests and by practical experience can be shown to be widely different in quality.

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WEATHER AND WHEAT YIELD IN WESTERN CANADA

II. INFLUENCE OF PRE-SEASONAL PRECIPITATION ON PLOT YIELDS
III. RELATION BETWEEN PRECIPITATION AND AGRICULTURAL YIELD[1]

BY J. W. HOPKINS[2]

Abstract

In continuance of a previous statistical study, the correlation between plot yields of wheat grown at experimental stations in central and southern Saskatchewan and Alberta and the amount of precipitation during the autumn, winter and spring months prior to sowing was investigated.

There was a significant relation between pre-seasonal precipitation and the yield secured from year to year on both the fallowed and stubble plots of a summerfallow-wheat-wheat rotation, above-average moisture being associated with increased yields. The annual yields of Marquis wheat from more fertile summerfallowed varietal test plots were not, however, significantly correlated with pre-seasonal precipitation, nor was there any consistent relation between this weather factor and the relative yield of certain early, medium-early and late-maturing varieties.

The annual average yield of wheat per acre from 1916–34 in three central and in three southern crop districts of Saskatchewan and Alberta showed a significant positive correlation with the available statistics of rainfall between May 1 and July 31. Yields in the southern districts were also positively correlated with pre-seasonal precipitation, whereas those in the central districts were not. The degree of association (R = 0.74, central; and 0.79, southern) was not adequate for the practical forecasting of annual production, but may be improved by refinements dependent on the accumulation of additional observational data.

Introduction

In a previous communication (8), the author reported a statistical study of the relation between annual variations in rainfall and temperature during the growing season and the yield of wheat from certain plots at agricultural experiment stations in central and southern Alberta and Saskatchewan. Three series of crop records were employed, permitting the correlation of the weather factors mentioned with (i) the yield of Marquis wheat grown in variety test plots; (ii) the relative order of yield of the wheat varieties Garnet (early maturing), Reward (early maturing), Marquis (medium-early) and Red Fife (late maturing); and (iii) the yield from the summerfallowed and stubble plots of a summerfallow-wheat-wheat rotation.

A significant relation between yield and the amount and distribution of seasonal rainfall was demonstrable from the first and third of these series. On the whole, above-average rainfall was associated with higher yield, though the result of a given increment of rain at a specified time seemed to be partly dependent on soil conditions. It was also concluded that the influence of weather conditions was not exerted mainly during a few "critical periods", but extended throughout the growing season.

An appreciable proportion of the recorded variation in yield was thus accounted for, but there remained in all series large residual variations. Although these undoubtedly arose in part from the fact that, at each station, the crop was grown on different soil each year, it was felt that the amount of precipitation during the autumn, winter and spring months might also have appreciably affected crop growth. The investigation has therefore been amplified by the inclusion of this factor in each of the three series of crop and weather correlations. An account of the results thus obtained is given in the first part of the present paper.

Following these studies of plot data, some preliminary consideration is given to the problem of determining the relation between weather conditions and agricultural yields by crop districts. The ultimate objective here is the formulation of regression or prediction equations which will enable a reasonable estimate of the size of a forthcoming crop to be made from a knowledge of the weather conditions affecting its development.

II. Influence of Pre-seasonal Precipitation on Plot Yields

A. Total Pre-seasonal Precipitation (Rain and Snow)

The total amounts of precipitation recorded each season, from August 1 of the preceding year to the date sixteen days before the sowing of the crop, for the various stations and seasons for which yield data were available for the previous study (8)*, are shown in Table I. The figures given include both rain and snow, but are expressed as "inches of rain", the customary conversion factor of 1/10 having been used to obtain the rain equivalent of snowfall.

TABLE I

PRE-SEASONAL PRECIPITATION (RAIN AND SNOW), EXPRESSED AS INCHES OF RAIN

Crop year	Edmonton	Lacombe	Lethbridge	Indian Head	Swift Current	Scott	Rosthern	Saskatoon*
1931	5.90	7.22	6.30	4.53	6.69	5.73	8.20	6.94
1930	5.71	5.80	8.37	8.90	5.34	5.29	7.32	4.23
1929	7.16	7.53	6.86	3.13	5.15	3.69	5.95	5.68
1928	8.30	12.01	13.43	11.32	9.11	7.76	6.57	7.47
1927	13.03	17.49	11.87	12.42	10.56	7.95	7.54	9.88
1926	8.35	14.17	9.98	7.51	7.64	6.79	8.78	6.89
1925	10.29	11.16	11.08	13.44	10.24	10.42	9.70	10.42
1922	5.88	4.96	7.42	13.99	—	4.36	6.92	7.45
1921	8.62	7.54	5.59	9.89	—	9.19	10.09	11.55
1920	11.75	10.79	12.63	11.85	—	8.98	8.27	8.89

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In order to specify the average effect of variations in previous rain and snow on yield, the partial regression, after allowing for the influence of succeeding rainfall during the growing season, was determined in each case. Utilizing previous arithmetical working, the calculation of each of these coefficients was accomplished in three stages as follows:

* *Mr. J. Patterson, Director of the Meteorological Service of Canada, kindly co-operated in this connection by supplying records not available in published form.*

(i) The seasonal variance of pre-seasonal precipitation and its covariance with yield were first calculated directly from the observational data. (ii) Then the regression of pre-seasonal precipitation on the amount and distribution of rainfall during the growing season, as specified by the rainfall distribution coefficients of the previous paper (8), was determined. Knowing now the crude variance and covariance of yield and pre-seasonal precipitation, and the regression of both yield (8) and pre-seasonal precipitation on the rainfall coefficients for the growing season, the final step (iii) was to compute the variance and covariance of the yield and pre-seasonal precipitation residuals, and from these the partial regression of yield on pre-seasonal precipitation. Since the three series of crop records were of unequal length, it was necessary to follow through the complete procedure for each series separately.

Pre-seasonal Precipitation and Yield of Marquis Wheat

(i) The method of calculating the seasonal variance and covariance of the crop and meteorological data has already been explained (8, page 312). Varietal test plot yields of Marquis wheat for ten years at each of seven stations have also been published (8, Table I).

From the data in Table I of the present paper the sum of the squares of the seasonal deviations of the corresponding pre-seasonal precipitation from the station averages was found to be 499.3909. In the same way these and the foregoing data gave 633.8319 as the sum of the products of the annual fluctuations of yield and pre-seasonal precipitation.

(ii) The regression coefficients β_0, β_1, ... β_5 of pre-seasonal precipitation p on the distribution coefficients ρ_0, ρ_1, ... ρ_5 of rainfall during the growing season were determined from the Normal Equations given below, of which the numerical coefficients of the left-hand side are derived as previously (8, page 312), and those of the right-hand side are the sums of the products of the seasonal deviations of p and the rainfall distribution coefficients ρ_0, ρ_1, ... ρ_5 (8, Table IV) respectively.

$$16.91616\beta_0 + .704273\beta_1 - 7.374067\beta_2 - .153974\beta_3$$
$$+ 3.366082\beta_4 + 2.677181\beta_5 = 42.56497$$

$$.704273\beta_0 + 13.445682\beta_1 - .966389\beta_2 - 5.327921\beta_3$$
$$+ .238760\beta_4 + 2.090910\beta_5 = -15.60284$$

$$-7.374067\beta_0 - .966389\beta_1 + 15.382760\beta_2 + 4.591087\beta_3$$
$$- 3.442486\beta_4 - 2.087548\beta_5 = -25.84757$$

$$- .153974\beta_0 - 5.327921\beta_1 + 4.591087\beta_2 + 16.260811\beta_3$$
$$+ .035470\beta_4 - 3.460086\beta_5 = 3.38522$$

$$3.366082\beta_0 + .238760\beta_1 - 3.442486\beta_2 + .035470\beta_3$$
$$+ 18.600138\beta_4 + .981074\beta_5 = 18.96055$$

$$2.677181\beta_0 + 2.090910\beta_1 - 2.087548\beta_2 - 3.460086\beta_3$$
$$+ .981074\beta_4 + 13.885332\beta_5 = 9.24139 \text{(I)}$$

These equations gave the following values for the unknowns:

$$\beta_0 = \quad 2.16962 \qquad\qquad \beta_3 = \quad .00902$$
$$\beta_1 = -1.37242 \qquad\qquad \beta_4 = \quad .52178$$
$$\beta_2 = - \ .56712 \qquad\qquad \beta_5 = \quad .33365$$

The corresponding regression coefficients α of yield on the rainfall coeffi‑cients were (8, p. 213)

$$\alpha_0 = \quad 5.16387 \qquad\qquad \alpha_3 = \quad .80204$$
$$\alpha_1 = -3.74079 \qquad\qquad \alpha_4 = -.67708$$
$$\alpha_2 = -6.74085 \qquad\qquad \alpha_5 = -.93891$$

(iii) It was not necessary to calculate the individual deviations of yield and pre-seasonal precipitation from the regression formulas. The required sums of squares and products of the residuals may be deduced from the sums prior to fitting the regressions by means of the identities (VI) and (VIa) of (8). In the present notation,

$$S(p - \beta_0\rho_0 - \beta_1\rho_1 - \ldots\ldots -\beta_5\rho_5)^2 = S(p^2) - \beta_0 S(p\rho_0)$$
$$- \beta_1 S(p\rho_1) - \ldots\ldots - \beta_5 S(p\rho_5) \ldots\ldots\ldots\ldots\ldots \text{(II)}$$
$$S(p - \beta_0\rho_0 - \beta_1\rho_1 - \ldots\ldots -\beta_5\rho_5)(y - \alpha_0\rho_0 - \alpha_1\rho_1 - \ldots\ldots - \alpha_5\rho_5)$$
$$= S(py) - \alpha_0 S(p\rho_0) - \alpha_1 S(p\rho_1) - \ldots\ldots -\alpha_5 S(p\rho_5) \ \text{(IIa)}$$

the individual quantities p, ρ and y (yield) being understood to be the annual deviations from the respective station averages.

Applying (II), the sum of the squares of the pre-seasonal precipitation residuals was calculated to be 357.9626. In the same way, 196.6834 was obtained for the sum of the products of the yield and pre-seasonal precipi‑tation residuals by means of (IIa). These gave $\gamma = 0.55$ bushels per acre as the average increase in yield associated with each additional inch of pre-seasonal precipitation. The sum of the squares of the yield residuals (8, Table V) was 6370.15. Table II shows the proportion of this total accounted for by the regression on pre-seasonal precipitation; the mean square due to the regression is actually slightly smaller than the mean square deviation, indicating that the regression coefficient γ is statistically insignificant. Accordingly, it cannot be concluded that there was any consistent relation between pre-seasonal precipitation and yield in this series of data.

TABLE II

ANALYSIS OF RESIDUAL VARIANCE OF YIELD OF MARQUIS WHEAT FROM TEST PLOTS

Variance due to	Degrees of freedom	Sum of squares	Mean square
Regression on pre-seasonal precipitation	1	108.07	108.07
Deviations from regression	56	6262.08	111.82
Total residual	57	6370.15	—

Pre-seasonal Precipitation and Differential Yield of Wheat Varieties

Annual varietal test yields of the four wheat varieties previously mentioned, at each of seven stations for the period 1925–31, have already been tabulated (8, Table II).

(i) The sum of the squares of the seasonal variations in pre-seasonal precipitation from the various station averages for this period was 349.2600. Summing the products of these variations and those of the four varietal yield differences previously studied gave the following values: Garnet-Reward, 125.310; Garnet-Marquis, −13.546; Garnet-Red Fife, 144.283; Marquis-Red Fife 187.070.

(ii) The Normal Equations determining the regression coefficients of pre-seasonal precipitation on the rainfall distribution coefficients for the growing season were:

$$12.441809\beta_0 + .447835\beta_1 - 4.232000\beta_2 + 1.855544\beta_3$$
$$+ 2.699285\beta_4 + 5.111341\beta_5 = 17.35489$$

$$.447835\beta_0 + 9.186527\beta_1 - .422194\beta_2 - 3.748035\beta_3$$
$$+ .565771\beta_4 + .077764\beta_5 = -5.28687$$

$$4.232000\beta_0 - .422194\beta_1 + 10.456264\beta_2 + .260742\beta_3$$
$$- 4.267647\beta_4 - 3.509379\beta_5 = -5.95805$$

$$1.855544\beta_0 - 3.748035\beta_1 + .260742\beta_2 + 7.905838\beta_3$$
$$- .955070\beta_4 - 1.870688\beta_5 = 6.72658$$

$$2.699285\beta_0 + .565771\beta_1 - 4.267647\beta_2 - .955070\beta_3$$
$$+ 16.362681\beta_4 + 1.468324\beta_5 = 8.62844$$

$$5.111341\beta_0 + .077764\beta_1 - 3.509379\beta_2 - 1.870688\beta_3$$
$$+ 1.468324\beta_4 + 10.407955\beta_5 = 3.86242 \text{ (III)}$$

whence, by means of the determinantal ratios previously used (8, Table VI)

$$\beta_0 = 2.67315 \qquad \beta_3 = -.73721$$
$$\beta_1 = -1.35145 \qquad \beta_4 = .31405$$
$$\beta_2 = .02076 \qquad \beta_5 = -.21058$$

The previously deduced (8, page 315) regression coefficients α of the varietal yield differences on $\rho_0, \rho_1 \ldots \rho_5$ are collected together in Table III.

TABLE III

REGRESSION COEFFICIENTS OF VARIETAL YIELD DIFFERENCES ON RAINFALL DISTRIBUTION COEFFICIENTS

Regression coefficient	Garnet-Reward	Garnet-Marquis	Garnet-Red Fife	Marquis-Red Fife
α_0	2.23029	.34679	2.98575	1.40547
α_1	−1.21200	1.63877	− .31845	−1.56621
α_2	1.93438	−1.07564	− .45215	−2.69321
α_3	− .69475	2.61538	− .79706	−2.19339
α_4	.07248	2.33236	4.25054	.92497
α_5	− .44483	.39608	.64787	1.08334

(iii) Applying (II), page 000, the sum of the squares of the pre-seasonal precipitation residuals was calculated to be 245.8330. The sums of products of the yield difference and pre-seasonal precipitation residuals, obtained by means of (IIa) were: Garnet-Reward, 77.222; Garnet-Marquis, −93.295; Garnet-Red Fife, −41.043; Marquis-Red Fife, 83.844. These gave partial regression coefficients of yield difference on pre-seasonal precipitation of 0.31, −0.38, −0.17 and 0.34 bushels per acre, respectively, per additional inch.

Table IV shows the extent to which the variance of the yield difference residuals is accounted for by the regression. The degree of correlation indicated is in all cases insignificant.

TABLE IV

ANALYSIS OF RESIDUAL VARIANCE OF VARIETAL YIELD DIFFERENCES

Variance	Garnet-Reward	Garnet-Marquis	Garnet-Red Fife	Marquis-Red Fife
Sum of squares:				
Due to regression on pre-seasonal precipitation (1 deg. freedom)	24.26	35.41	6.85	28.60
Deviations from regression (35 degrees of freedom)	493.92	1686.38	2097.20	856.34
Total residual (36 degrees of freedom)*	518.18	1721.79	2104.05	884.94
Mean square:				
Due to regression on pre-seasonal precipitation	24.26	35.41	6.85	28.60
Deviation from regression	14.11	48.18	59.92	24.47

* From (8), Table VII.

Pre-seasonal Precipitation and Yield of Wheat from Summerfallowed and Stubble Land

Wheat yields secured from year to year at the stations maintaining this three-course experimental rotation have already been listed (8, Table III).

TABLE V

REGRESSION COEFFICIENTS OF YIELD FROM SUMMER-FALLOWED AND STUBBLE LAND ON RAINFALL DISTRIBUTION COEFFICIENTS

Regression coefficient	Summer-fallowed land	Stubble land	Difference, summer-fallow −stubble
α_0	9.80077	9.09916	.70161
α_1	− .48082	−2.34024	1.85942
α_2	−3.87536	−2.37545	−1.49991
α_3	.00891	.83645	− .82754
α_4	− .70476	−1.61300	.90824
α_5	.19378	.03891	.15488

(i) The required sum of the squares of annual fluctuations in pre-seasonal precipitation was found to be 354.5470. Products of these variations and those of the yield from summerfallowed land, of the yield from stubble land and of the difference in yield from summerfallow and stubble totalled 764.690, 653.652 and 111.038 respectively.

(ii) The previously determined (8) regression coefficients α of yield on the rainfall distribution coefficients ρ for the growing season are given in Table V. The Normal Equations specifying the regression coefficients β of pre-seasonal precipitation on the rainfall distribution coefficients were:

$$12.843475\beta_0 - .335858\beta_1 - 4.365599\beta_2 + 1.826327\beta_3$$
$$+ 1.794788\beta_4 + 4.455203\beta_5 = 33.15616$$

$$- .335858\beta_0 + 8.950589\beta_1 - .005577\beta_2 - 4.254751\beta_3$$
$$+ 1.254894\beta_4 + .280159\beta_5 = - 9.52226$$

$$-4.365599\beta_0 - .005577\beta_1 + 9.856559\beta_2 + 2.416812\beta_3$$
$$- 4.985248\beta_4 - .724501\beta_5 = -15.06180$$

$$1.826327\beta_0 - 4.254751\beta_1 + 2.416812\beta_2 + 10.123139\beta_3$$
$$- .217015\beta_4 - .367921\beta_5 = 3.12835$$

$$1.794788\beta_0 + 1.254894\beta_1 - 4.985248\beta_2 - .217015\beta_3$$
$$+ 15.041716\beta_4 + 1.515575\beta_5 = 7.77009$$

$$4.455203\beta_0 + .280159\beta_1 - .724501\beta_2 - .367921\beta_3$$
$$+ 1.515575\beta_4 + 10.644241\beta_5 = 10.05162 \text{(IV)}$$

From these

$$\beta_0 = 2.67315 \qquad \beta_3 = -.73721$$
$$\beta_1 = -1.35145 \qquad \beta_4 = .31405$$
$$\beta_2 = .02076 \qquad \beta_5 = -.21058$$

(iii) Proceeding as before, the sum of the squares of the pre-seasonal precipitation residuals was found to be 255.3420. The residual sums of products of pre-seasonal precipitation and yield were: summerfallowed land, 380.286; stubble land, 303.421; difference between summerfallowed and stubble land, 76.865. The partial regression coefficients γ of yield on pre-seasonal precipitation were therefore; summerfallowed land, 1.49; stubble land, 1.19; and difference between summerfallow and stubble, 0.30 bushels per acre for each additional inch of pre-seasonal precipitation. Analyses of variance, shown in Table VI, indicated that there was a significant degree of association between pre-seasonal precipitation and the yield from either summerfallowed or stubble

TABLE VI

ANALYSIS OF RESIDUAL VARIANCE OF YIELD FROM SUMMERFALLOWED AND STUBBLE LAND

Variance	Summer-fallow	Stubble	Summer-fallow −stubble
Sum of squares:			
Due to regression on pre-seasonal precipitation (1 degree of freedom)	566.37	360.55	23.14
Deviations from regression (35 degrees of freedom)	1931.92	1970.72	1543.51
Total residual (36 degrees of freedom)*	2498.29	2331.27	1566.65
Mean square:			
Due to regression on pre-seasonal precipitation	566.37	360.55	23.14
Deviation from regression	55.20	56.31	44.10

From (8), Table XI.

land.   Annual variations in the difference in yield between the fallowed and stubble areas, however, evidently were not related consistently to this meteorological factor.

B. PRE-SEASONAL RAINFALL ONLY

Studies at the Swift Current experimental station extending over a period of seven years are reported by Barnes and Hopkins (1) to have indicated no appreciable increase in soil moisture from the presence of snow cover.   This they attribute to the fact that moisture can enter the soil only if the soil temperature is above freezing-point.   Such a condition may sometimes prevail in the autumn after the first snowfall, but usually frost follows quickly and the soil becomes frozen before absorbed moisture, derived from melted snow, can penetrate to any appreciable depth.

In these circumstances it might be thought that variations in the amount of winter snowfall from year to year would have little effect on subsequent crop yields, and might in fact merely obscure any real correlation between yield and autumn and spring rainfall.   Although this seemed not to be so in the case of the qualitative character, protein content, previously investigated (9), it was nevertheless thought desirable to determine whether the three series of crop yields studied in the preceding section were more closely correlated with pre-seasonal rainfall alone than with the total of pre-seasonal rain and snow.   Accordingly, the annual quantities of pre-seasonal precipitation given in Table I were separated into rain and snow, and the amounts of rain entered in Table VII.

TABLE VII

PRE-SEASONAL PRECIPITATION (RAIN ONLY) IN INCHES

Crop year	Edmonton	Lacombe	Lethbridge	Indian Head	Swift Current	Scott	Rosthern	Saskatoon
1931	3.07	5.42	3.72	2.32	4.73	3.17	3.80	3.36
1930	3.27	3.06	3.83	2.87	2.11	2.44	2.20	1.80
1929	3.63	3.71	2.33	1.10	1.34	0.93	1.95	2.41
1928	3.22	4.95	5.61	6.19	5.83	4.41	5.12	4.25
1927	7.50	10.23	8.35	5.55	4.76	5.05	3.68	3.32
1926	4.53	9.67	7.92	4.00	2.65	3.92	6.70	5.03
1925	4.17	5.98	5.03	5.69	6.31	5.21	7.10	4.67
1922	2.36	2.68	3.66	8.98	—	2.32	4.13	5.58
1921	4.06	4.95	2.16	6.53	—	5.81	6.09	5.84
1920	3.33	5.56	4.14	3.56	—	4.24	5.22	4.72

In estimating the partial regression of each series of wheat yields on pre-seasonal rainfall, the procedure employed in the preceding section was again followed, with the substitution of the rainfall indicated in Table VII for the corresponding amount of total precipitation shown in Table I.

Pre-seasonal Rainfall and Yield of Marquis Wheat

(i) Seasonal covariation of yield and pre-seasonal rainfall gave rise to a sum of products of 419.993.   The sum of the squares of fluctuations in rainfall amounted to 229.4541.

(ii) The left-hand side of the Normal Equations to determine the regression coefficients β_0', β_1', ... β_5' of pre-seasonal rainfall p' on the summer rainfall coefficients ρ_0, ρ_1, ... ρ_5 remained as in (I). It was necessary however to replace the numerical quantities on the right-hand side by the sums of products generated by the seasonal covariation of p' and ρ_0, ρ_1, ... ρ_5, namely 26.57925, -6.18413, -7.89021, 9.28615, 3.57478 and 1.69060. Making this substitution, the regression coefficients β' were found to be:

$$\beta_0' = 1.65587 \qquad \beta_3' = .43480$$
$$\beta_1' = -.36322 \qquad \beta_4' = -.08300$$
$$\beta_2' = .10801 \qquad \beta_5' = -.01235$$

(iii) These calculations provided the data necessary for the adjustment of the crude sums of squares and products of yield and pre-seasonal rainfall by means of (II) and (IIa) to give the corresponding functions of the residuals. The residual pre-seasonal rainfall sum of squares was 180.3284, and the residual sum of products of yield and pre-seasonal rainfall 203.541. The partial regression coefficient γ' of yield on pre-seasonal rainfall was therefore 1.13 bushels per acre for each additional inch of total pre-seasonal precipitation.

Table VIII shows the distribution of the residual yield variance after fitting the regression. The mean square due to the regression now exceeds the

TABLE VIII

ANALYSIS OF RESIDUAL VARIANCE OF YIELD OF MARQUIS WHEAT FROM TEST PLOTS

Variance due to	Degrees of freedom	Sum of squares	Mean square
Regression on pre-seasonal rainfall	1	229.74	229.74
Deviations from regression	56	6140.41	109.65
Total residual	57	6370.15	—

mean square deviation, but the ratio of these corresponds to a z value (7, Sec. 47) of only 0.370, whereas the 5% point for 1 and 56 degrees of freedom is approximately 0.700. It must be concluded therefore, that although there was some association between pre-seasonal rainfall and yield in the sample of seasons examined, it is not improbable that this was an entirely chance effect.

Pre-seasonal Rainfall and Differential Yield of Wheat Varieties

(i) For this series, the sum of the squares of the annual variations in pre-seasonal rainfall was 174.4978. The sums of the products of the variations in pre-seasonal precipitation and in the four yield-differences were: Garnet-Reward, 53.451; Garnet-Marquis, -46.686; Garnet-Red Fife, 13.205; Marquis-Red Fife, 90.234.

(ii) The sums of products of p' and ρ_0, ρ_1, ... ρ_5, to be substituted in the right-hand side of Normal Equations (III) were 17.35065, -6.18374,

-3.33279, 4.83174, 1.77350, and 3.69845. These resulted in the sub-joined values of β':

$$\beta_0' = 1.75868 \qquad\qquad \beta_3' = -.35544$$
$$\beta_1' = -.88800 \qquad\qquad \beta_4' = -.08418$$
$$\beta_2' = .16442 \qquad\qquad \beta_5' = -.49808$$

(iii) Employing these coefficients in (II) and (IIa) as before gave 142.7492 for the sum of the squares of the residual deviations of pre-seasonal rainfall, and for the residual sums of products of pre-seasonal rainfall and the yield differences: Garnet-Reward, 18.580; Garnet-Marquis, -64.392; Garnet-Red Fife, -48.159; Marquis-Red Fife 52.138. The partial regression coefficients γ' of the yield-differences on pre-seasonal rainfall were therefore 0.13, -0.45, -0.34 and 0.37 bushels per acre per inch respectively. As is shown by the

TABLE IX

ANALYSIS OF RESIDUAL VARIANCE OF VARIETAL YIELD DIFFERENCES

Variance	Garnet-Reward	Garnet-Marquis	Garnet-Red Fife	Marquis-Red Fife
Sum of squares:				
Due to regression on pre-seasonal rainfall (1 degree of freedom)	2.42	29.05	16.25	19.04
Deviations from regression (35 degrees of freedom)	515.76	1692.74	2087.80	865.90
Total residual (36 degrees of freedom)	518.18	1721.79	2104.05	884.94
Mean square:				
Due to regression on pre-seasonal rainfall	2.42	29.05	16.25	19.04
Deviation from regression	14.74	48.36	59.65	24.74

analysis of variance in Table IX, these are all statistically insignificant, and provide no evidence of any consistent effect of variations in the amount of pre-seasonal rain on the relative yield of the four varieties of wheat.

Pre-seasonal Rainfall and Yield of Wheat from Summerfallowed and Stubble Land

(i) In this case, the sum of the squares of the annual deviations from the station averages of pre-seasonal rainfall was 193.5744. The corresponding sums of products of yield and pre-seasonal rainfall were: summerfallowed land, 488.182; stubble land, 452.653; difference, summerfallow—stubble, 35.529.

(ii) Summing the products of p' and ρ_0, ρ_1, ... ρ_5 gave 21.14056, -2.90190, -2.57160, 4.67500, -3.01081 and 1.27903. Substituting these in Equations (IV), the following regression coefficients β^1 were found:

$$\beta_0' = 2.15063 \qquad\qquad \beta_3' = -.23254$$
$$\beta_1' = -.30911 \qquad\qquad \beta_4' = -.15812$$
$$\beta_2' = .61592 \qquad\qquad \beta_5' = -.71546$$

(iii) The residual pre-seasonal rainfall sum of squares, deduced as before, was 150.3218, and the residual sums of products of yield and pre-seasonal rainfall were 267.216, 238.575 and 28.641. These yielded the following partial regression coefficients γ': summerfallowed land, 1.78; stubble land 1.59; and difference summerfallow-stubble 0.19 bushels per acre for each additional inch of pre-seasonal rain.

It will be seen by reference to Table X that the variance of yield from both the fallowed and stubble land, accounted for by the regression, significantly exceeded the mean square deviation, the z values being 1.053 and 0.958 respectively (5% point = 0.707). No significant differential effect of pre-seasonal rain on the yield from summerfallow and stubble was demonstrable.

TABLE X

ANALYSIS OF RESIDUAL VARIANCE OF YIELD FROM SUMMERFALLOWED AND STUBBLE LAND

Variance	Summer-fallow	Stubble	Summer-fallow −stubble
Sum of squares:			
Due to regression on pre-seasonal rainfall (1 degree of freedom)	475.01	378.64	5.46
Deviation from regression (35 degrees of freedom)	2023.28	1952.63	1561.19
Total residual (36 degrees of freedom)	2498.29	2331.27	1566.65
Mean square:			
Due to regression on pre-seasonal rainfall	475.01	378.64	5.46
Deviation from regression	57.81	55.79	44.61

The regression coefficients γ' of yield on pre-seasonal rainfall are slightly larger than the coefficients γ previously obtained with the total of pre-seasonal rain and snow as the independent variable, suggesting that precipitation in the form of rain was more efficient in augmenting yield than was a corresponding amount of snow. On the other hand, they accounted for a somewhat lower proportion of the yield variance, indicating that the correlation between pre-seasonal precipitation and yield was higher when the total of both rain and snow was considered. The difference is not, however, statistically significant, having regard to the number of observations. There may have been some additional effect of snow, but on the whole it seems probable that the major part of any increase in yield resulting from pre-seasonal precipitation was attributable to autumn or spring rainfall.

Significant effects of this nature may be of considerable agricultural importance. As previously intimated, soil conditions in the plots subjected to this experimental grain rotation probably approximated fairly closely to those met with in general farming practice. The varietal test plots on the other hand constituted a special case, and a comparison of the two sets of meteorological correlations throughout the investigation is indicative of the manner in which cultural practices may modify the incidence of weather effects.

III. Relation Between Precipitation and Agricultural Yields

As was pointed out elsewhere (8, Section 1), any detailed analysis of the relation between weather conditions and agricultural yields would require a series of records more extensive than any that have so far come to the author's attention. However, the results of the study of plot yields had indicated that the amounts of precipitation prior to and during the growing season were likely to be the most important individual weather factors, although the possibility of significant non-linear temperature effects should not be overlooked (2, 10). It was felt, therefore, that a brief examination of the correlation of the former with some available agricultural yield data might provide some indication of the degree of accuracy likely to be attained by a simple yield prediction equation.

For the purpose of crop reporting, the prairie provinces have each been divided into a number of crop districts. The annually reported yields of wheat in six of these districts, three in the central and three in the southern regions of Saskatchewan and Alberta, were selected for study. These districts centered on Lacombe, Scott and Saskatoon, and on Calgary, Swift Current and Regina respectively. Records of the average annual wheat yield by crop districts were available as far back as the year 1908, but the boundaries of the districts as originally constituted later underwent considerable modification. For this reason, it was judged that only the data from 1916 onwards were sufficiently homogeneous for correlational purposes.

The average wheat yield for each district is computed by the provincial Departments of Agriculture from estimates supplied by farmers in the various townships included, supplemented by threshermen's returns. Consequently, the figures given may be affected to some extent by subjective bias. It is probable however that the limitations of the meteorological data with which these were correlated introduced a greater source of error, since for four of the six districts continuous records at a single station only were available. The magnitude of local variations in the incidence of rainfall is now under investigation. In the meantime it must be suspected that observations at one or two points did not represent accurately the average conditions prevailing in different years over the areas in question.

It has been shown by Fisher (6) that the distribution of the multiple correlation coefficient R, in samples from populations of actually zero association, has a positive bias specified by the ratio of the number of independent variables involved to the number of residual degrees of freedom. High correlations between several variables, deduced from short series of observations, may be expected therefore to be the outcome in part at least of chance, rather than truly causal, association, and to require modification in the light of subsequent experience. It is not clear that this circumstance was taken into consideration by Bogue (3) who has recently reported high correlations between weather and wheat yield in the prairie provinces generally.

As the crop yields now under consideration comprised only 19 years' records it was clearly undesirable in view of the foregoing considerations, rein-

forced as they were by the doubtful adequacy of the meteorological data, to attempt any detailed correlation of yield and seasonal precipitation. The latter was therefore specified merely by the total amounts received during the preceding period from August 1 to April 30 inclusive, and during the period of active crop growth (May 1 to July 31) each year.

The regression of yield on pre-seasonal and summer precipitation, thus defined, was first determined for each of the six districts individually. It was found that there was in all cases a significant correlation between precipitation and the yield recorded, that there were no statistically significant differences between the results for the three central districts or between those for the three southern ones, but that the residual deviations were relatively large. Accordingly, the correlation between yield and precipitation in the annual averages of the three central and the three southern districts, which would be somewhat less affected by random inaccuracies in the data, was next examined.

Both series of yield and precipitation averages are given in Table XI. In arriving at the total pre-seasonal precipitation, the conventional con-

TABLE XI

AVERAGE WHEAT YIELD (BU. PER ACRE) AND PRECIPITATION (INCHES OF RAIN) IN
CENTRAL AND SOUTHERN DISTRICTS

Year	Central Districts			Southern Districts		
	Yield	Precipitation		Yield	Precipitation	
		Preceding, Aug. 1– Apr. 30	May 1– July 31		Preceding, Aug. 1– Apr. 30	May 1– July 31
1916	18.0	6.08	9.61	16.3	8.33	8.34
1917	15.2	9.54	3.82	16.9	8.02	3.53
1918	11.8	7.29	4.44	10.3	7.09	3.67
1919	15.5	6.94	4.38	9.6	7.69	3.34
1920	15.4	9.97	5.46	18.6	8.65	7.78
1921	14.2	9.72	6.95	13.5	7.05	7.29
1922	13.8	6.23	4.13	20.4	8.64	6.91
1923	25.6	7.03	11.28	23.7	7.10	12.85
1924	8.1	7.67	3.66	16.0	7.38	6.24
1925	19.7	11.45	6.49	20.0	11.76	6.34
1926	15.8	9.98	6.74	19.5	9.22	7.27
1927	21.5	11.09	9.64	19.5	12.68	11.26
1928	22.8	8.63	8.51	24.7	11.28	8.80
1929	11.2	5.31	4.30	8.1	5.25	4.97
1930	16.6	5.74	5.40	12.7	8.00	4.80
1931	15.2	6.08	7.58	7.4	5.56	5.11
1932	16.2	9.70	6.76	14.4	9.78	8.49
1933	8.7	7.76	5.05	11.0	8.01	6.66
1934	10.2	7.72	6.63	7.4	9.45	5.75

version factor, 0.1, was used to estimate the rainfall equivalent of snow. It may be noted that annual variations in the average yield recorded were more pronounced in the southern than in the central districts.

Denoting by b_1 and b_2 the regression coefficients of yield on precipitation (x_1) during the period, May 1 to July 31 of the crop year, and on that (x_2)

during the period from August 1 of the preceding year to April 30 of the crop year respectively, the Normal Equations determining these were computed from the data for the central districts, shown in Table XI, to be:

$$86.3931 \; b_1 \; + \; 11.5969 \; b_2 \; = \; 140.005$$
$$11.5969 \; b_1 \; + \; 63.3356 \; b_2 \; = \; 42.670$$

giving $b_1 = 1.57 \pm 0.33$ and $b_2 = 0.39 \pm 0.39$ bushels per acre for each additional inch of precipitation.

For the southern district averages, the Normal Equations found were:

$$111.4669 \; b_1 \; + \; 36.3307 \; b_2 \; = \; 161.475$$
$$36.3307 \; b_1 \; + \; 66.8299 \; b_2 \; = \; 105.393$$

giving $b_1 = 1.14 \pm 0.40$ and $b_2 = 0.96 \pm 0.52$ bushels per acre for each additional inch of precipitation.

In both cases the regression on precipitation during the period May 1 to July 31 was significant, and of the same order, the difference between the two values of b_1 being statistically insignificant. The yields reported for the southern districts apparently were affected also by the amount of pre-seasonal precipitation, the coefficient b_2 being 1.85 times its standard error. This ratio would be expected to be attained on the average only about 8 times in 100 as a chance result. For the central districts, on the other hand, the coefficient b_2 does not exceed its standard error.

This more definite indication of dependence on pre-seasonal precipitation in the southern districts perhaps resulted in part from greater losses of soil moisture by evaporation during the late summer and autumn months. Thus for the six-year period 1925–30, the average amounts of evaporation from a free-water surface during August and September at Lacombe (central Alberta) and Swift Current (southern Saskatchewan) were calculated from annual data published in (4) and (5) to be:

	August	September
Lacombe	3.53 in.	2.14 in.
Swift Current	6.43 in.	3.82 in.

It might be supposed that rainfall during the summer months (May to July) of the year previous to cropping exerts a sensible influence on yield, through mosture conserved in the summerfallowed acreage; but this could not be established from the present data. Including this as a third independent variable, x_3, the Normal Equations determining the regression coefficients became:

$$86.3931 \; b_1 \; + \; 11.5969 \; b_2 \; - \; 13.7365 \; b_3 \; = \; 140.005$$
$$11.5969 \; b_1 \; + \; 63.3356 \; b_2 \; - \; 3.6792 \; b_3 \; = \; 42.670$$
$$-13.7365 \; b_1 \; - \; 3.6792 \; b_2 \; + \; 89.7921 \; b_3 \; = \; -36.701$$

and

$$111.4669 \; b_1 \; + \; 36.3307 \; b_2 \; + \; 23.9322 \; b_3 \; = \; 161.475$$
$$36.3307 \; b_1 \; + \; 66.8299 \; b_2 \; + \; 6.8354 \; b_3 \; = \; 105.393$$
$$23.9322 \; b_1 \; + \; 6.8354 \; b_2 \; + 111.7377 \; b_3 \; = \; 73.387$$

which gave $b_1 = 1.54$, $b_2 = 0.38$, $b_3 = -0.16$ bushels per acre for the central district averages and $b_1 = 1.05$, $b_2 = 0.97$, $b_3 = 0.37$ bushels per acre for the southern district averages for each additional inch of rain during the periods specified. In both cases the regression of yield on the previous summer's rain was insignificant, as is shown by the analyses of variance in Table XII.

TABLE XII

ANALYSIS OF VARIANCE OF AVERAGE WHEAT YIELD IN CENTRAL AND SOUTHERN DISTRICTS

Variance	Degrees of freedom	Central Districts		Southern Districts	
		Sum of squares	Mean square	Sum of squares	Mean square
Accounted for by regression on precipitation during May 1–July 31, and preceding Aug. 1–Apr. 30	2	236.11	118.06	284.55	142.28
Additional effect of precipitation during May 1–July 31 of previous year	1	2.16	2.16	14.74	14.74
Residual	15	144.70	9.65	221.37	14.76
Total	18	382.97	—	520.66	—
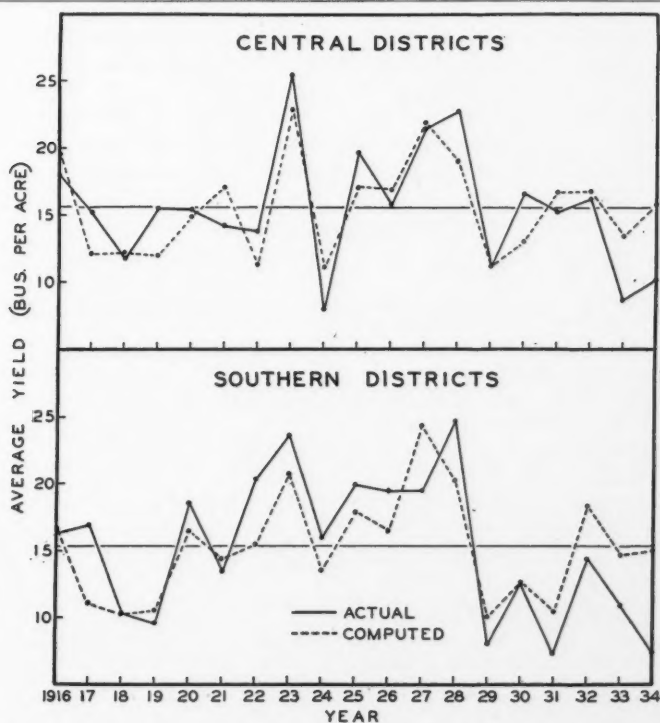
FIG. 1. *Actual and computed yields of wheat in three central and three southern crop districts. Horizontal lines indicate average yield, 1916–34.*

Reverting to the previously determined regression of yield on x_1 and x_2 only, the coefficient of multiple correlation R between average precipitation and yield was 0.74 for the central districts and 0.79 for the southern ones. Fig. 1 illustrates the agreement between the average yields actually reported and those calculated (Y) from the regression equations

$$Y = \quad\ \ 2.41 + 1.57x_1 + 0.39x_2$$
$$Y = -0.63 + 1.14x_1 + 0.96x_2$$

for the central and southern districts respectively.

If this degree of reliability could be maintained in the estimation of future crops, prediction equations even of this simple nature might be of some use particularly in view of the early date (end of July) at which estimates could be made. Nevertheless, the accuracy attained still leaves a good deal to be desired. The standard deviation of the actual from the calculated yields, as computed from the mean square residual (16 degrees of freedom) was 3.0 bushels per acre for the central and 3.8 for the southern district averages, and as the area annually devoted to wheat growing in the prairie provinces is of the order of 20,000,000 acres, the total production estimated in this way will undoubtedly be greatly in error in some seasons. It remains to be seen whether future refinements, such as the accumulation of meteorological records at an increased number of stations in each district, the determination of the limits of effective rainfall, and the incorporation of additional weather variables (when the series of annual yields has become sufficiently numerous) will bring this uncertainty within the limits of practical application.

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STUDIES ON THE HETEROPHYID TREMATODE, *APOPHALLUS VENUSTUS* (RANSOM, 1920) IN CANADA

PART I. MORPHOLOGY AND TAXONOMY[1]

BY THOMAS W. M. CAMERON[2]

Abstract

This minute intestinal trematode occurs in the cat, dog, racoon and heron in the lower Ottawa Valley. Its morphology is redescribed and its nomenclature reconsidered. It is believed to be specifically distinct from the closely related form, *A. donicus* of Central Europe. *A. brevis* is recorded from the loon.

Introduction

The Heterophyidae is a family of very small Trematoda living in the small intestine of a wide variety of vertebrates. In recent years considerable attention has been given to this group as its members appear to show a very low degree of host specificity and to possess the ability of developing to functional maturity in any fish-eating bird or mammal, including the common laboratory animals. A considerable number of species has been recorded from man and most parasitologists believe that all are potential human species. Comparatively few life histories have been worked out, but in such as have, two intermediate hosts have been found to be essential— a snail and a fish.

The Heterophyidae live on the mucosa of the small intestine and until recently have been regarded as comparatively harmless worms. The recent investigations of Africa, Garcia and de Leon in the Philippines, however, have shown that, potentially at least, they may be very serious parasites indeed. These workers performed 108 almost consecutive autopsies in the Manila city morgue and discovered nine cases of intestinal infestations with Heterophyidae, four different species being involved. The cause of death in these nine cases was found to be cardiac trouble (six), malaria, lobar pneumonia and meningitis (one each). The high proportion of cardiac cases suggested some causal relationship with the parasites, and investigation showed that such was the case. The trematodes are characteristically covered with minute, backwardly directed spines; they live between the villi

of the small intestine, and the spines must serve, in conjunction with body movements, to keep them in very close contact with the mucosa.   Presumably land mammals are not "natural" fish-eaters, and therefore not so completely adapted to the flukes as are aquatic mammals and fish-eating birds, and so the trematodes may actually penetrate the deeper layers of the mucosa. The eggs, in some cases at least, are unable to pass into the lumen of the intestine, and because of their situation, gain entrance to the general circulation by way of the lacteals or venules of the villi, and are carried to various parts of the body.   In the six cases mentioned, they had reached the smaller vessels of the heart where they had caused emboli, with fatal results.

The discovery of a member of the Heterophyidae common in the Montreal district, carried by fresh-water fish, is accordingly, of considerable interest.

In connection with the survey of internal parasites of animals in Canada, which is being conducted by the Institute, eggs of a species of Heterophyid— later found to be a species of *Apophallus*—were found to be common in cats in Ste. Anne de Bellevue, Quebec.   With the permission and assistance of the Dominion and Provincial Departments of Fisheries, a large number of local fish was obtained and fed, by species, to non-infected cats, which had been bred at Macdonald College and which had not previously been fed on fish. In every case, with the exception of cats fed on Rock Bass, infections with the same species of *Apophallus* developed.
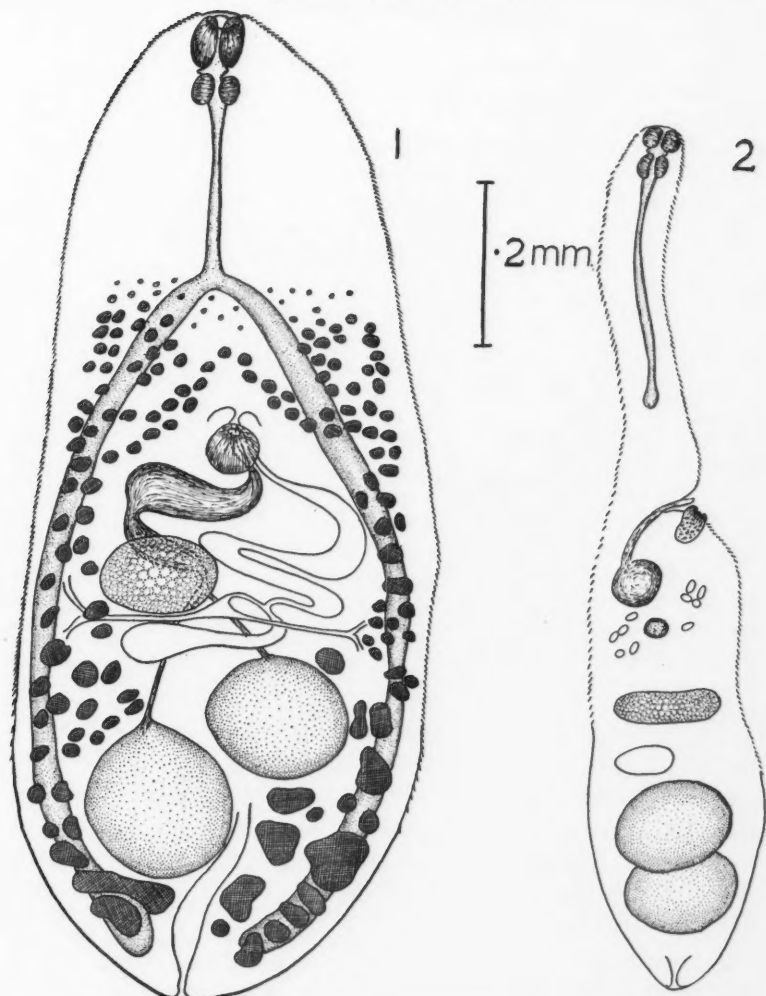
Apophallus venustus (Ransom, 1920)

Adults

The contour of the body is elongated oval in most cases, being slightly broader at the posterior end (Fig. 1).   In many cases however, it becomes narrower in the middle, resembling the sole of a child's shoe; in a few cases it is almost pyriform.   These variations in outline are found in batches fixed, stained and mounted in bulk and so subjected to identical conditions of technique.   It varies in length from 0.95 to 1.4 mm. and its maximum breadth is from 0.25 to 0.55 mm.   The mean size is approximately 1.1 by 0.4 mm.   The anterior half is only about half the thickness of the posterior half.

The cuticle is provided with numerous small spines in the anterior half. At the level of the genital pore they commence to decrease in number and the posterior quarter of the body is almost entirely free.

The oral sucker is slightly sub-terminal and ventral, and is oval in shape, measuring 0.07 mm. long by 0.06 mm. broad.

The ventral sucker is in the mid-line of the body just in front of the mid-point.   It is slightly but distinctly muscular: in size, it is slightly smaller than the oral sucker (0.06 mm.).   It is situated on the posterior margin of a genital sinus and is directed anteriorly.   The genital openings occur at the base of the sinus, anterior to the ventral sucker, and from their antero-lateral margins arise two feebly muscular papillae (Figs. 1 and 2), the so-called genital papillae.

Apophallus venustus
  Fig. 1.   *Typical adult drawn from ventral aspect.*
  Fig. 2.   *Medial view of adult, reconstructed from serial sections.*

Digestive System

The oral sucker is connected by a small prepharynx to the muscular pharynx. The oesophagus is long and simple and gives rise to two intestinal caeca, a considerable distance in front of the genital sinus and about a quarter of the body length from the anterior end. The caeca run to the posterior end of the body, terminating almost in contact with the excretory vesicle.

Male Genital System

There are two almost spherical testes, situated obliquely to each other in the posterior region of the body; the right testis is generally posterior. They have a diameter of 0.15 to 0.2 mm. The vasa efferentia unite at about the level of and dorsal to the ovary. The vas deferens quickly becomes enlarged to form a conspicuous seminal vesicle, which is roughly S-shaped with a central constriction and lies between the ovary and the genital sinus. It is continued by an ejaculatory canal to open in the base of the genital sinus on the same side as the ovary. There is no cirrus.

Female Genital System

The ovary is transversely ellipsoidal, measuring 0.15 mm. by 0.1 mm., with a smooth contour, and is situated anterior to both testes, on the same side of the body as the posterior one. Behind the ovary and towards the dorsal surface of the body, is a transversely oval spermatheca. Lying mainly on the side of the body opposite to that on which lies the ovary, but encroaching on the ovarian side of the body as well, is a relatively short uterus of several loops. It terminates at the base of the genital sinus, by the side of the male canal. Laurer's canal is absent.

The yolk follicles are numerous small groups of cells which occupy the posterior part of the body. Their anterior limit is about level with the junction of the two intestinal caeca, and is remarkably constant in all the specimens examined. The anterior follicles, which cross the mid-line of the body, are smaller and more numerous than the posterior follicles (Fig. 1.). The posterior follicles pass behind the testes and occupy all the otherwise unoccupied space there. The yolk ducts originate about the middle of the yolk glands, between the spermatheca and the ovary and unite to form a common yolk reservoir of varying size in the mid-line.

The eggs are few in number (up to 100) and measure 26 to 32μ by 18 to 22μ.

Excretory System

The excretory system was not studied in detail. The excretory vesicle is Y-shaped with the posterior stem S-shaped in order to pass between the two testes. The excretory pore is terminal.

Nomenclature

The systematic position of the various species in this genus has given rise, in the past few years, to a considerable amount of discussion.

The genus *Apophallus* was created in 1909, by Lühe, for a species from *Larus ridibundus* from Central Europe which had been misidentified in 1898 by Creplin as *Distomum lingua*, and renamed *Distomum mühlingi* by Jägerskiöld the following year. In the same year, Looss placed it in the genus *Tocotrema* and it was not until 1909 that Lühe made it the type of his new genus with the name of *Apophallus mühlingi* (Jägerskiöld, 1899) Lühe, 1909.

In 1920, Ransom (9) described a new species from *Larus delawarensis* (from Washington, D.C.) under the name of *Apophallus brevis* and created a

new genus, *Cotylophallus* to receive two other new species, *viz:*— *C. similis* (from *Phoca vitulina* from Washington, D.C.) and *C. venustus* (from dogs, cats and *Vulpes lagopus* from the same area). These four species were separated from each other on the disposition of the yolk follicles.

Meanwhile, in 1919, Skrjabin and Lindtrop (10) created the genus *Rossicotrema* to receive the new species *R. donicum* from dogs and cats in Russia. This work was inaccessible to Ransom at the time of writing and there is little doubt that if he had known of it, he would not have created his genus *Cotylophallus* but would have included his species in the genus *Rossicotrema:* they were transferred to this genus by Ciurea in 1924.

In 1929, in a revision of the family, Witenberg (11), after examination of a cotype of *A. brevis* Ransom, concluded that it also should be included in the genus *Rossicotrema*. He considered however that no specific differences existed among the four species now included in the genus and he suppressed all Ransom's species as synonyms of *R. donicum*. However, he recognized the genus *Apophallus* as distinct from *Rossicotrema*, mainly on the basis of the disposition of the testes, which were tandem to each other in the former and oblique in the latter. He regarded the genus *Tocotrema* as closely related to *Rossicotrema*, differing from it mainly in the presence of a single gonotyle (a name he applied to the genital papillae in these genera and analogous structures in other genera in this family), instead of a pair.

In the following year (1930) however, as the result of re-studying the available material, he concluded that the number of gonotyles was a specific rather than a generic character and he suppressed *Rossicotrema* in favor of *Tocotrema* (12). However, previous workers had suppressed the genus *Tocotrema* in favor of *Cryptocotyle;* Witenberg disagreed with this and recognized both genera as valid. This view has not been generally accepted and is especially criticized by Stunkard in his paper on the life-cycle of *Cryptocotyle lingua*. Witenberg had separated these two genera on the basis of shape of body and disposition of testes. Stunkard (1930) argued that the genera *Cryptocotyle* and *Tocotrema* were congeneric. He examined hundreds of *C. lingua* (which Witenberg had placed in *Tocotrema*) and found a few were as short and broad as *C. concava* (the type of *Cryptocotyle*), although ordinarily the body was longer and narrower. In a few specimens also, the testes lay at the same level, although normally they were diagonal. In *C. concava* however, the testes were on opposite sides of the median plane whereas in *C. lingua* both testes extended across this plane. He regarded these differences as specific rather than generic and referred both species to *Cryptocotyle*.

Price (7), in 1931, in a discussion on these species, concluded that *Cryptocotyle* and *Apophallus* were valid genera, separated from each other mainly in the structure of the ventral-sucker–genital-opening complex. In *Cryptocotyle* the genital sinus is a spacious, somewhat muscular structure, the ventral sucker is greatly reduced and is situated in the anterior wall of the sinus; the genital openings are behind the ventral sucker and the ducts open at the base of a *single* papilla-like gonotyle. In this genus he included *Tocotrema* Looss,

1899, as well as two other genera (*Hallum* Wigdor, 1918, and *Ciureana* Skrjabin, 1923) which are not discussed in this paper.

In *Apophallus*, the genital sinus is reduced in size and its walls feebly developed, while the ventral sucker, which is relatively strongly developed, is on its posterior margin; the genital openings are in front of the ventral sucker and two papilla-like gonotyles are present. In this genus he included *Rossicotrema* and *Cotylophallus*.

Four species of *Apophallus* were recognized by Price, *viz:*—
Body elongated, with more or less distinct constriction between ventral sucker and bifurcation of oesophagus:

Yolk glands extend to level of ventral sucker; oesophageal bifurcation about one-third body length from anterior end . . *A. mühlingi* (type).

Yolk glands do not extend anteriorly as far as ventral sucker: oesophageal bifurcation about one-fifth of body length from anterior end
. . . . *A. crami* Price, 1931.
(from *Larus californicus*, Oregon).

Body ovoid in shape; yolk glands extend to level of oesophageal bifurcation or slightly beyond . . . . *A. donicum*
(including *venustus* and *similis*).

Body elongated pyriform in shape; yolk glands extend only slightly beyond ventral sucker . . . . *A. brevis.*

In the following year (1932) he described a new species, *Apophallus zalophi* from *Zalophus californianus* (8). This species is related to *A. donicus* but is smaller and thicker, has a much longer pre-pharynx and relatively large yolk follicles, which do not pass posterior to the anterior edge of the testes. There is, moreover, a greater tendency for the testes to be opposed. (Ciurea (6) (1933) has removed this species from the genus *Apophallus* to make it the type of a new genus, *Pricetrema.*)

Ciurea (1933) did not agree with Price in regarding the genus *Rossicotrema* as synonymous with *Apophallus*, believing the differences in the shape of the body, the situation of the testes and the development of the metacercaria, to be of generic importance.

Moreover, he did not regard *R. donicum* as being identical with *R. venustum* or *R. simile*. He believed all three species were valid and that they could be distinguished by the position of the anterior limit of the yolk glands. In *R. venustum*, these reach almost to the level of the bifurcation of the intestine; in *R. simile*, they pass this point, while in *R. donicum*, they do not reach it. The other species were left in *Apophallus*.

In 1935, Africa and Garcia (1) described *Apophallus eccentricus* from the dog in the Philippines. In this species, however, not only is the pre-pharynx long, but the genital pore is *lateral* instead of anterior, the yolk glands are considerably post-acetabular and the uterus extends to the posterior region of the body. This latter point alone seems sufficient to exclude it from this genus.

We have to deal with one of four possible species, *R. donicum*, *A. brevis*, *C. venustus* and *C. similis*, which have been variously referred to three genera by modern authors, *viz.*:—

Witenberg (11, 12) (1929-1930) regards all four as synonymous and refers them all to the genus *Tocotrema* under the name of *T. donicum*.

Price (7) (1931) regards all four as belonging to the genus *Apophallus* considering the last two as synonymous with the first and so recognizing only two valid species, *A. donicus* and *A. brevis*.

In *A. donicus*, the body is ovoid and the yolk glands reach to the level of the oesophageal bifurcation, while in *A. brevis*, the body is pyriform with the yolk glands passing only slightly beyond the acetabulum.

Ciurea (6) (1933) regards all four as valid species and refers *A. brevis* to the genus *Apophallus* and the other three to the genus *Rossicotrema*. He separated these three species from each other as follows:

R. venustus—Yolk follicles reach almost to level of bifurcation of oesophagus.

R. simile—Yolk follicles pass in front of level of bifurcation of oesophagus.

R. donicum—Yolk follicles do not reach the level of bifurcation of oesophagus.

A considerable amount of this confusion is due to the fact that these three workers have adopted different generic criteria. Witenberg regards the relationship of the testes to each other as of fundamental importance; Price places emphasis on the anterior or posterior position of the ventral sucker relative to the genital openings; Ciurea considers not only the disposition of the testes, but the shape of the body and development of the trematode as important generic features, while considering the structure of the ventral-sucker–genital-sinus complex as of super-generic importance.

While Witenberg is possibly correct in regarding the presence of one or of two gonotyles as only of specific importance, it would seem that the opening of the genital ducts anterior or posterior to the ventral sucker is of very considerable importance, more so even than the relationship of the testes to each other. Accordingly, Price's criterion is accepted in this paper and *Tocotrema* regarded as a synonym of *Cryptocotyle*, and *Rossicotrema* as a synonym of *Apophallus*.

The question of species is much more difficult. There is a considerable similarity not only among the four "species" mentioned above, but among them and *A. crami* and *A. mühlingi*. They are differentiated on characters which appear to vary within wide limits.

Outline of Body

As mentioned above, the outline in most of my specimens is an elongated oval, somewhat more pointed at the anterior end than at the posterior, and slightly broader at the level of the testes than at the level of the oesophageal bifurcation. In a few cases, the body is markedly broader at the level of the testes and the body approaches the pyriform: in others the width at these two points is approximately similar, but there is a distinct constriction

between them (Fig. 3). The majority are ovoid however, and the two excep-
tions do not reach the limits shown in Price's drawing of *A. crami*, or Ransom's
drawing of *A. brevis*. It may well be the case that all the specimens of these
two species have constant body outlines, but the fact that this is not the case
in the Canadian material greatly reduces the value of this feature as a specific
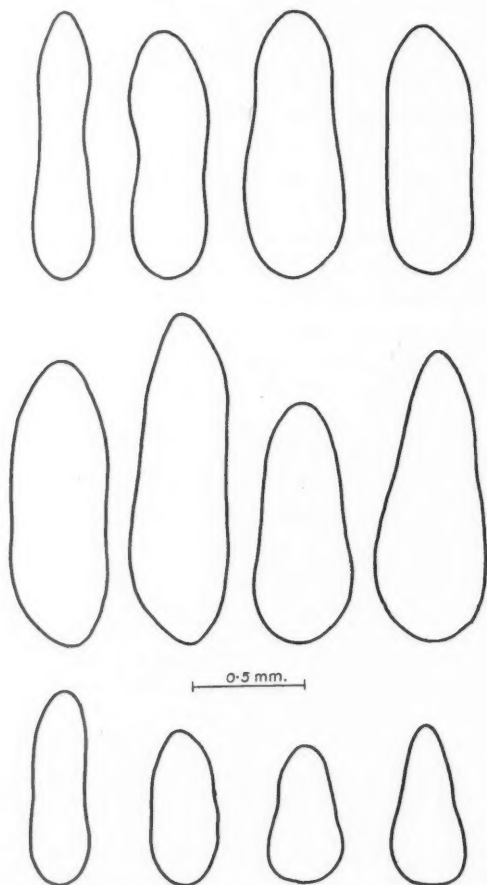character.

FIG. 3.   *Camera lucida drawings, all to same scale, of A. venustus (upper two rows) and
A. donicus (bottom row).*

Testes

In every case (except the obvious malformations mentioned later in this
series), the testes are distinctly obliquely placed with regard to each other.

Yolk Glands

In all the Canadian specimens the yolk glands reach to about the level of the oesophageal bifurcation, which is placed about the junction of anterior and second quarters of the body. These yolk glands always meet across the middle line at this point.

There is a uniformity about all the drawings of *A. mühlingi* published. All have elongated forms, with the testes almost, but not quite tandem and the yolk glands ceasing at the level of the ventral sucker. In *A. crami*, the same elongated body form is found with the testes more obviously oblique and the yolk glands ceasing well posterior to the ventral sucker.

In *A. donicus* (in Ciurea's various drawings as well as in a number of specimens he kindly sent to me) there is considerable variation in body form (Fig. 3). The typical outline in expanded specimens is elongated oval, but contracted specimens are distinctly pyriform. In the former, the testes are oblique; in the latter they are almost side-by-side. However, the specimens show that the yolk glands terminate about the level of the ventral sucker and Ciurea also has drawn attention to this fact in his various papers on the subject (Plate 1, Figs. 1 and 2).

In *A. venustus*, on the other hand, which is otherwise similar to *A. donicus*, the yolk glands reach to the level of the oesophageal bifurcation (Plate I, Figs. 4, 5, 6). *R. simile* as described by Ransom, does not appear to differ specifically from this form, while *A. brevis* approaches very close to *R. donicum*.

I suggest dividing these species as follows:—

1. Yolk glands do *not* reach level of ventral sucker . . . . . *A. mühlingi.*
   A. donicus.
   A. brevis.

In this group, *A. mühlingi* has a long narrow body with the testes almost tandem; in *A. donicus* the body is oval to pyriform with the testes oblique; in *A. brevis*, the body is elongated pyriform and the testes oblique.

2. Yolk glands reach level of oesophageal bifurcation . . . . *A. venustus* (including *C. similis.*)

Minor differences between *venustus* and *donicus* lie in the testes. In the former they are always distinctly oblique, more or less spherical and fairly posterior: in the latter, they tend towards a side-by-side arrangement, although generally distinctly oblique, are more or less triangular in outline and occupy a slightly more anterior position in the body. *A. donicus* has a maximum length of 1.14 mm. and width of 0.46 mm.: *A. venustus* has a maximum length of 1.4 mm. and width of 0.55 mm. The eggs of *A. donicus* are slightly larger than those of *A. venustus* (35-40 by 19–24μ and 26–32 by 18–22μ respectively).

Accordingly, I agree with Price in regarding *similis* as a synonym of *venustus*, but not in regarding *venustus* as a synonym of *donicus*. The specimens from Canada accordingly are referred to the species *Apophallus venustus*. As will

be seen in the second part of this paper, there are certain biological differences between the American and European forms which further support this separation.

Apophallus venustus has only been recorded from eastern North America in Washington, D.C., and the lower Ottawa Valley, P.Q. It has been found in the following hosts: Dog, cat, racoon (*Procyon lotor*) Alaskan fox (*Vulpes lagopus*), harbour seal (*Phoca vitulina*) and the great blue heron (*Ardea herodias herodias*). The Alaskan fox came from the National Zoological Park in Washington City.

Apophallus donicus in the restricted sense used in this paper has been found in Europe from the following hosts, naturally or after feeding on infected fish: Dog, cat, rabbit, white mouse, *Vulpes lagopus* and *Mustela sarmatica*. It has also been recorded by Ciurea from the following birds: *Mergus merganser, Nycticorax nycticorax, Buteo buteo, Ciconia ciconia, Larus ridibundus, Sterna cantiaca* (the specimens in the two last-mentioned hosts were non-ovigerous), *Asio otus, Coturnix communis, Turtur communis* and *Columba livia* (the last four hosts mentioned were experimentally infected).

In addition to the specimens found in cats, a considerable number of specimens of *Apophallus* was recovered from a young loon (*Gavia immer*). The loon had only been a few days in the district and it is almost certain that its infection was not acquired locally. These specimens differ from the cat specimens in a number of points. The oesophagus is longer—about a third of the body length—and the yolk glands never reach to the bifurcation (Plate I, Fig. 3) but cease at the level of the ventral sucker. This point was quite constant in the 30 specimens examined. In all, the testes are obliquely placed to each other and are both rather more anterior than in the cat specimens.

There is considerable variation in the shape of the body, however. In some specimens this is elongated oval, but in most it lies between elongated pyriform and elongated with a more or less distinct constriction between the ventral sucker and bifurcation of the oesophagus. Using Price's criteria, some specimens could be called *A. mühlingi* and others, *A. brevis*. As however, in European specimens of *A. mühlingi* the testes are almost tandem to each other and in the present specimens they are always distinctly oblique, it would seem that it should more correctly be referred to *A. brevis*.

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PLATE I

FIGS. 1 AND 2. *Apophallus donicus (from specimens from dog received from Professor Ciurea, Roumania).*

FIG 3. *Apophallus brevis (from loon, Canada).*

FIGS. 4–6. *Apophallus venustus (from cat, Canada).*
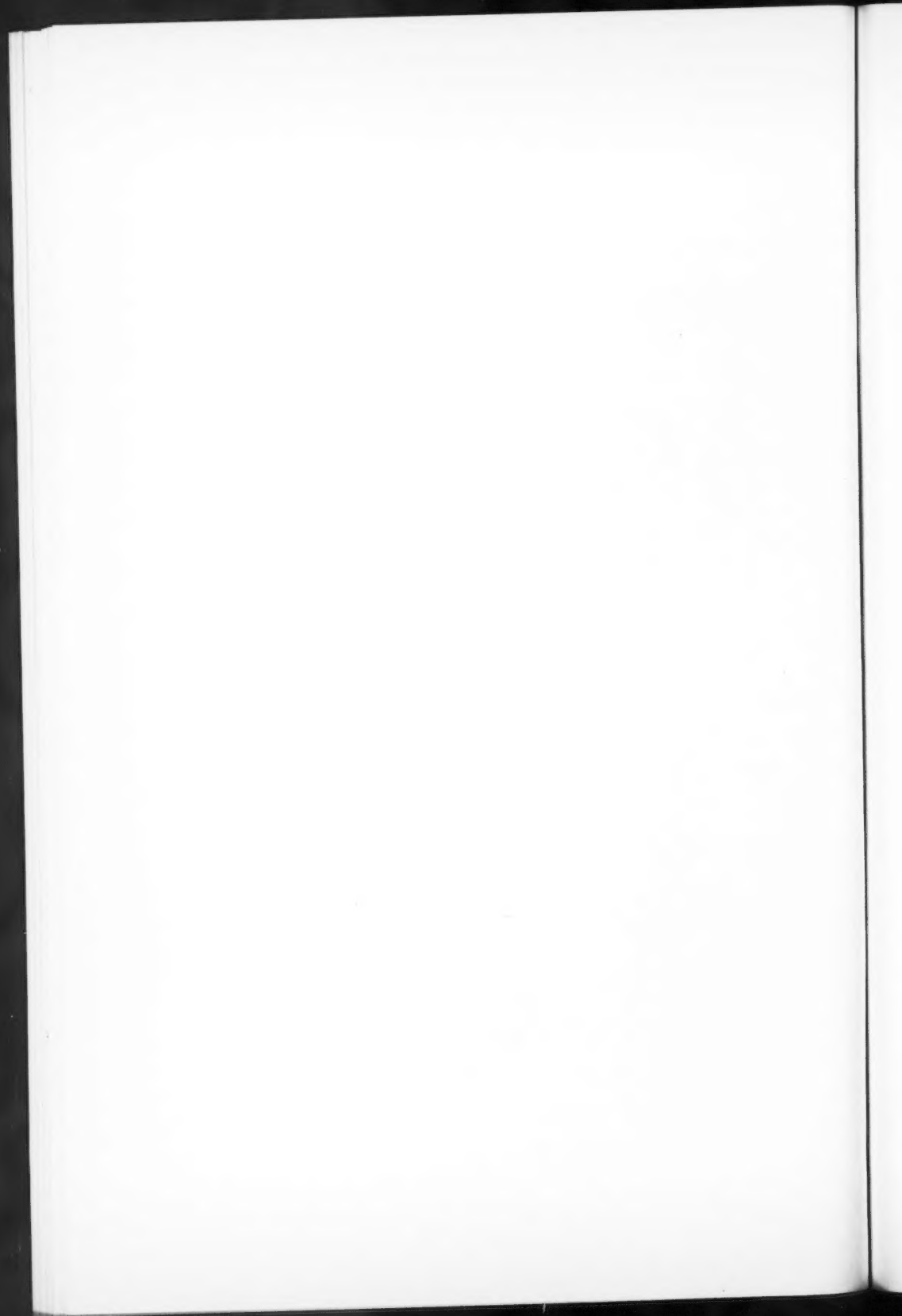
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